# 中醫藥基礎與臨床研究之趨勢

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# 前　言

- 中醫藥淵遠流長，沿用數千年，融入於國人日常生活之中（治病、養生）
- 實際經驗累積，建立完整中醫藥學體系及典籍著作（本草、內經、難經、傷寒論...）
- 但缺乏現代化之科學研究。因此有提倡：
  - 中西醫結合（大陸）
  - 中西醫整合醫學（台灣）

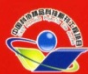
# 前　言

- 大陸：中西醫結合學會
  - 中國中西醫結合雜誌（中文版）
  - Chinese Journal of Integrated Traditional and Western Medicine
    - 專家論壇、臨床論著、實驗研究、臨床經驗
  - 中國結合醫學雜誌（英文版）
  - Chinese Journal of Integrative Medicine
    - Original articles, experimental research, clinical experiences
- 台灣：中西整合醫學會
  - 中西整合醫學雜誌（中文版）
  - Journal of Integrated Chinese and Western Medicine
    - 凡中西醫整合有關之論著









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# CHINESE JOURNAL OF INTEGRATIVE MEDICINE

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# 中西整合醫學雜誌

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中華民國九十一年十二月

# 中醫藥治療焦慮症之研究

## Clinical and Experimental studies on Anxiety Treated with Traditional Chinese Medicine

# 前　言

- 焦慮和失眠是人類普遍存在的一種困擾，每個人多少都有過親身經歷。
- 尤以最近金融海嘯，受失業之苦，放無薪假，股市下跌，致內心煩躁、焦慮、不安與失眠，求診中西醫患者有增加的趨勢。

# 焦慮症(Anxiety)之病因

- 中醫並無”焦慮症”之名，
  - 從臨床症狀看，屬於情志病範疇，
  - 其病因與”心悸”、”驚悸”、”怔忡”、”不寐”等病有關。

# 心悸・驚悸

- 心悸，是指患者自覺心臟跳動，心慌不安，它包括驚悸和怔忡。
- 因驚而悸者謂之驚悸，多屬陣發，發病與情緒有關，時作時止，病情輕。

# 怔忡・不寐

- 怔忡即無所觸動而悸者，發作無時，持續不已，病情重。驚悸日久可發展為怔忡。
- 不寐是指經常性失眠之病證。

# 焦慮症之辨證論治

- 依張學斌等將焦慮症之病因病機，分為下列八型(陝西中醫函授,5.2001)

1. 心神不寧：善恐易驚,坐臥不安
   - 治則：養心安神,鎮驚定志
   - 方用：
     - 平補鎮心丹(和劑局方)：酸棗仁、茯苓、麥冬、五味子…。
     - 酸棗仁湯。

2. 腎精不足：恐懼緊張,惶惶不安,虛煩盜汗

- 治則：補腎益精定志
- 方用： 六味地黃丸

3. 氣血不足：善恐不安,氣短,自汗無力,面色蒼白

- 治則：補益氣血,安神
- 方用：遠志丸加減

4. 肝膽不足：緊張恐懼,坐臥不安,伴兩肋不適

- 治則：補益肝膽
- 方用：補膽防風湯加減：人參、茯神、防風、細辛、川芎、 獨活、 前胡、 大棗

5. 心膽氣虛：緊張不安，坐臥不寧，氣短，面色蒼白

- 治則：益氣，溫膽，化痰
- 方用：四君子湯合溫膽湯加減

6. 肝鬱血虛：善恐，緊張，胸脇脹滿，虛煩易怒

- ◈ 治則：養血舒肝，理氣，寧神
- ◈ 方用：丹梔逍遙散

7. 痰火擾心：驚恐不安，性急多言，躁狂，頭昏，頭痛，口苦，舌紅

- ◈ 治則：清心豁痰
- ◈ 方用：黃連溫膽湯

## 8. 心火旺盛

- 善驚意亂；口舌生瘡，口渴欲冷飲
- 治則：清心瀉火
- 方用：導赤散加減(加黃連,酸棗仁,黃芩)

# 酸棗仁湯治療焦慮症之臨床評估

1. 材料：酸棗仁湯(金匱要略方)：

   虛勞虛煩不得眠之著名古方。

   - 酸棗仁、茯苓、川芎、知母、甘草(7.5:2:2:1:1) 1 Kg，於5000 ml 95% alcohol室溫浸泡3天後，於80℃水浴抽取，過濾。於60℃真空濃縮，冷凍乾燥，通過80號篩，裝膠囊。

2. 病人：

1) 在內科門診randomize病人

2) 年齡20~60歲

3) 男•女

4) 病人必須符合下列者始納入研究:

① 病人必須有sympathetic hyperactivity , motor hypertonicity及apprehension.

② 病人Morbid Anxiety Inventory(MAI) ，Score在14~30.

③ 病人無特殊情況(心衰竭、高血壓、懷孕)及二週前未服中西藥者。

④ 經中醫診斷辨證心神不寧型。

3. 焦慮症臨床評估:

1) 依MAI (Score14~30)評估焦慮症

2) 依Hamilton Anxiety Scale (HAS)評估焦慮改善情形

3) 依Digital Symbol Substitution Test (DSST)評估心理性運動作用(psychomotor effect)

# 病態焦慮表 (Morbid Anxiety Inventory; Salkind 1972)

1. 一般性焦慮症Generalized Anxiety Disorder
   - 常感到不安、易怒、煩躁
   - 容易疲勞
   - 覺得肌肉緊繃(背、頸、肩膀)
   - 不容易維持記憶力
2. 社會畏懼症 Social Phobia
   - 害怕陌生人的場合
   - 害怕公開演說
   - 害怕見到陌生人
   - 盡可能遠離恐懼情境

# 病態焦慮表(Morbid Anxiety Inventory; Salkind 1972)

3. 恐慌性疾患 (Panic Disorder)
   - 心悸、心跳加快
   - 流汗
   - 感覺呼吸困難
4. 曠野恐慌症 (Agoraphobia)
5. 特別性畏懼症 (Specific Phobia)
6. 創傷後壓力症 (Post-traumatic Stress Disorder)

# Hamilton Anxiety scale

| 1.焦慮的情緒 Anxious Mood | 無 | 輕微 | 中等 | 嚴重 | 極嚴重 |
|---|---|---|---|---|---|
| (1)擔憂 | □ | □ | □ | □ | □ |
| (2)預期恐慌 | □ | □ | □ | □ | □ |
| 2.緊張 Tension | | | | | |
| (1)驚嚇 | □ | □ | □ | □ | □ |
| (2)易哭 | □ | □ | □ | □ | □ |
| (3)容易疲勞 | □ | □ | □ | □ | □ |
| 3.害怕 Fear | | | | | |
| (1)怕黑暗 | □ | □ | □ | □ | □ |
| (2)怕陌生人 | □ | □ | □ | □ | □ |
| (3)怕獨處 | □ | □ | □ | □ | □ |
| 4.失眠 Insomnia | | | | | |
| (1)難以入眠 | □ | □ | □ | □ | □ |
| (2)多夢 | □ | □ | □ | □ | □ |
| (3)夢中驚醒、惶恐不安 | □ | □ | □ | □ | □ |
| 5.智力的 Intellectual | | | | | |
| (1)無法集中精神 | □ | □ | □ | □ | □ |
| (2)記憶力不好 | □ | □ | □ | □ | □ |

# Hamilton Anxiety scale

| | | | | | |
|---|---|---|---|---|---|
| 6.憂鬱的情緒 (Depressed Mood ) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 7.身體肌肉方面(Somatic Complaints Muscular) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 8.身體感覺方面 (Somatic Complaints Sensory) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 9.心血管系統的症狀(Cardiovascular Symptoms) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 10.呼吸系統的症狀 (Respiratory Symptoms) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 11.腸胃系統的症狀(Gastrointestinal Symptom) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 12.泌尿系統的症狀 (Genitourinary Symptoms) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 13.自主神經系統症狀(Autonomic Symptoms) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 14.會談時的舉止 (Behavior at Interview) | ☐ | ☐ | ☐ | ☐ | ☐ |
| 15.生理上的行為(Physiological Behavior) | ☐ | ☐ | ☐ | ☐ | ☐ |

# Digital Symbol Substitution Test(DSST)
# 數字符號取代試驗

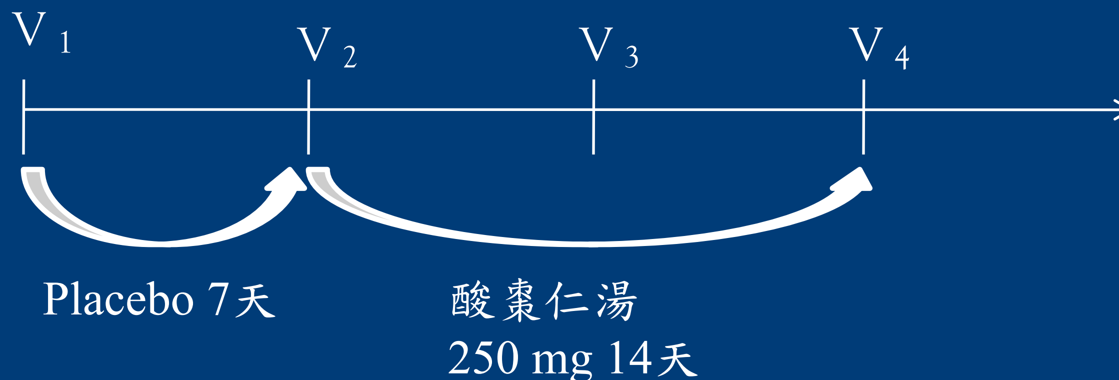
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| — | ⊥ | ㄈ | L | ㄩ | ○ | ⌒ | × | ＝ |

| 2 | 1 | 3 | 7 | 2 | 4 | 8 | 1 | 5 | 4 | 2 | 1 | 3 | 2 | 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | |

| 9 | 5 | 8 | 4 | 7 | 3 | 6 | 5 | 1 | 9 | 3 | 8 | 7 | 6 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | |

## 4. Protocol

門診次數




$V_1$:

1.建立個人史及檢查資料

2.量血壓及心博率

3.MAI, HAS及DSST評估表

$V_2$:

1.量血壓及心博率

2.MAI, HAS及DSST評估表

$V_3$ 、$V_4$同$V_2$

## 5.結果

(1)全部42位病人，完整研究有36位病人；有6位被剔除(1人有placebo effect，3人不願意，2人不來門診)

(2)服用酸棗仁湯2週後對於焦慮症狀及DSST皆有改善

# Effects of STZ on MAI, HAS and DSST

| | | Before the 1st week | After the 1st week | After the 2st week | After the 3st week |
|---|---|---|---|---|---|
| MAI | Mean | 27.4 | 26.2 | 22.9 | 23.9 |
| | SD | 7.1 | 8.5 | 9.8 | 7.9 |
| P value | | | > 0.4 | < 0.001 | > 0.4 |
| HAS | Mean | 23.7 | 21.9 | 14.2 | 17.7 |
| | SD | 6.9 | 7.3 | 8.2 | 7.0 |
| P value | | | > 0.1 | < 0.001 | < 0.05 |
| DSST | Mean | 39 | 42 | 50 | 45 |
| | SD | 12 | 11 | 10 | 12 |
| P value | | | > 0.1 | < 0.01 | < 0.05 |

Studies on the Suanzaorentang in the treatment of anxiety (Psychopharmacology, 85; 486-487, 1985)

# 酸棗仁對於小鼠抗焦慮作用之研究
# Anxiolytic effect of Seed of Ziziphus jujuba in mouse models of anxiety

(Peng WH, Hsieh MT et al. J Ethnopharmacol 72, 435-441, 2000)

1. 黑白室
2. 舉臂式十字迷宮

酸棗 *Ziziphus jujuba* Mill. var. *spinosa* (Bge.) Hu ex H. F. Chou

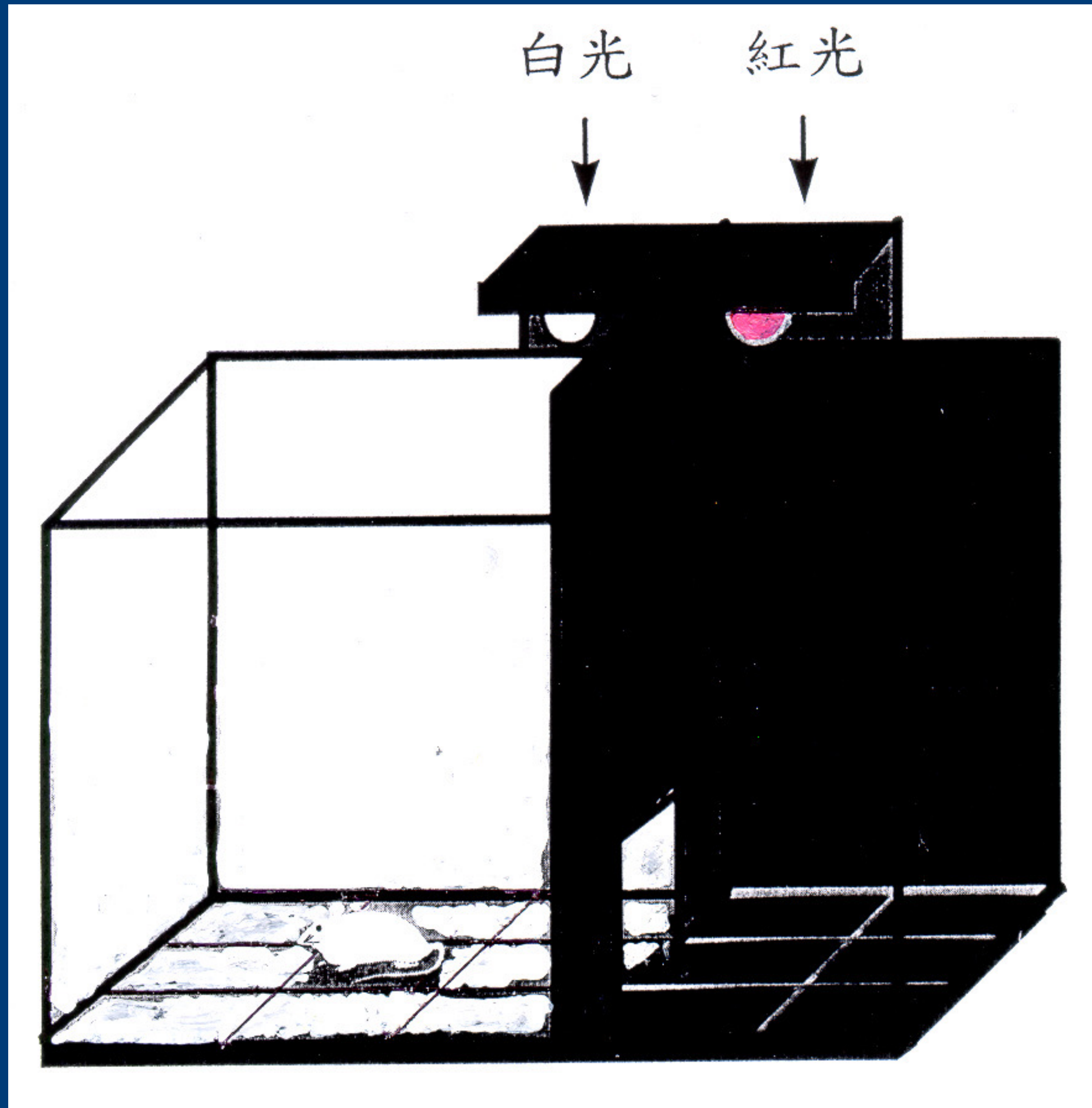


# 1. 黑白室

## Black/white test

(Sanchez, Pharmacol & Toxicol, 1995)

- 依囓齒類動物害怕紅光及白光的天性設計之模式


白光
紅光

- 評估指標
  - 記錄小鼠停留在黑、白室的總時間
- 焦慮與抗焦慮之評估標準

| 評估標準 | 焦慮 | 抗焦慮 |
| --- | --- | --- |
| 黑室滯留時間 | ↑ | ↓ |
| 白室滯留時間 | ↓ | ↑ |

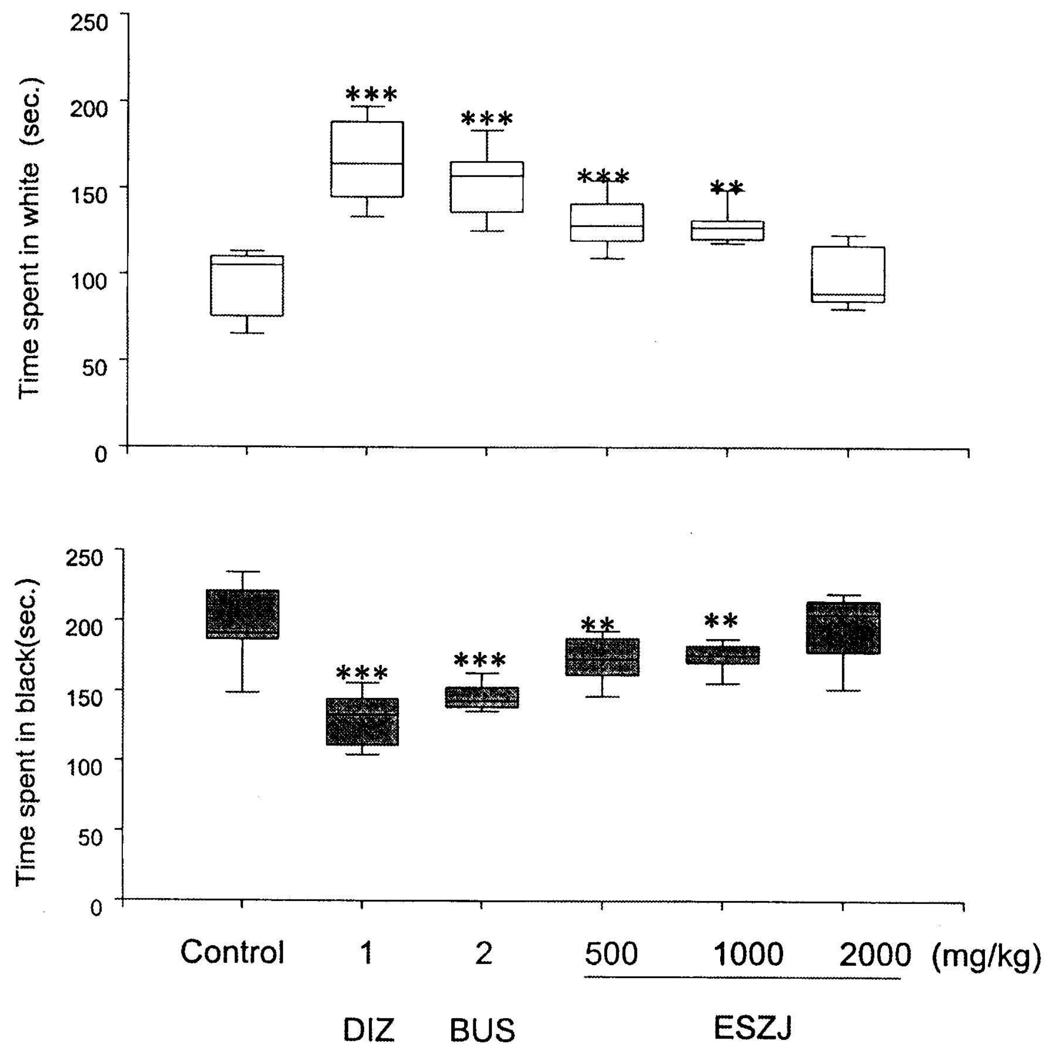



Fig. 2. Effects of diazepam (DIZ), buspiron (BUS) and ethanol extracts of SZ (ESZJ) on the time spent in white and black chambers in the black and white test in mice. $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with the control group (Kruskal-Wallis H following by Mann–Whitney $U$-test).

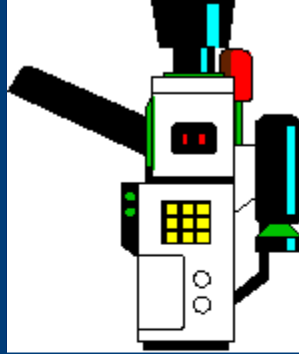
# 2. 舉臂式十字形迷宮

## (Elevated Plus-maze)

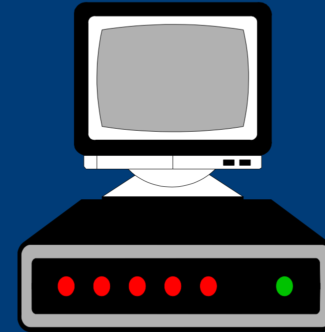
( Lister, J Neurosci Method , 1985)

- 依據嚙齒類動物厭惡高處及開放空間的天性而設計

攝影機

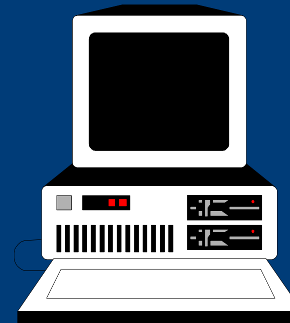




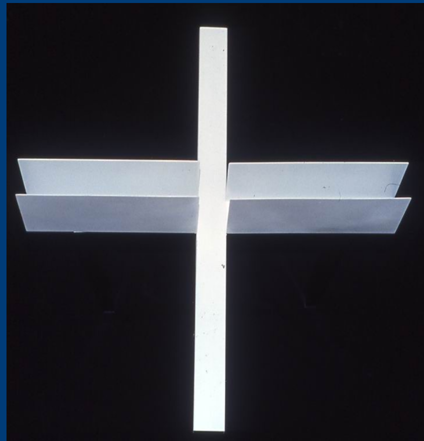


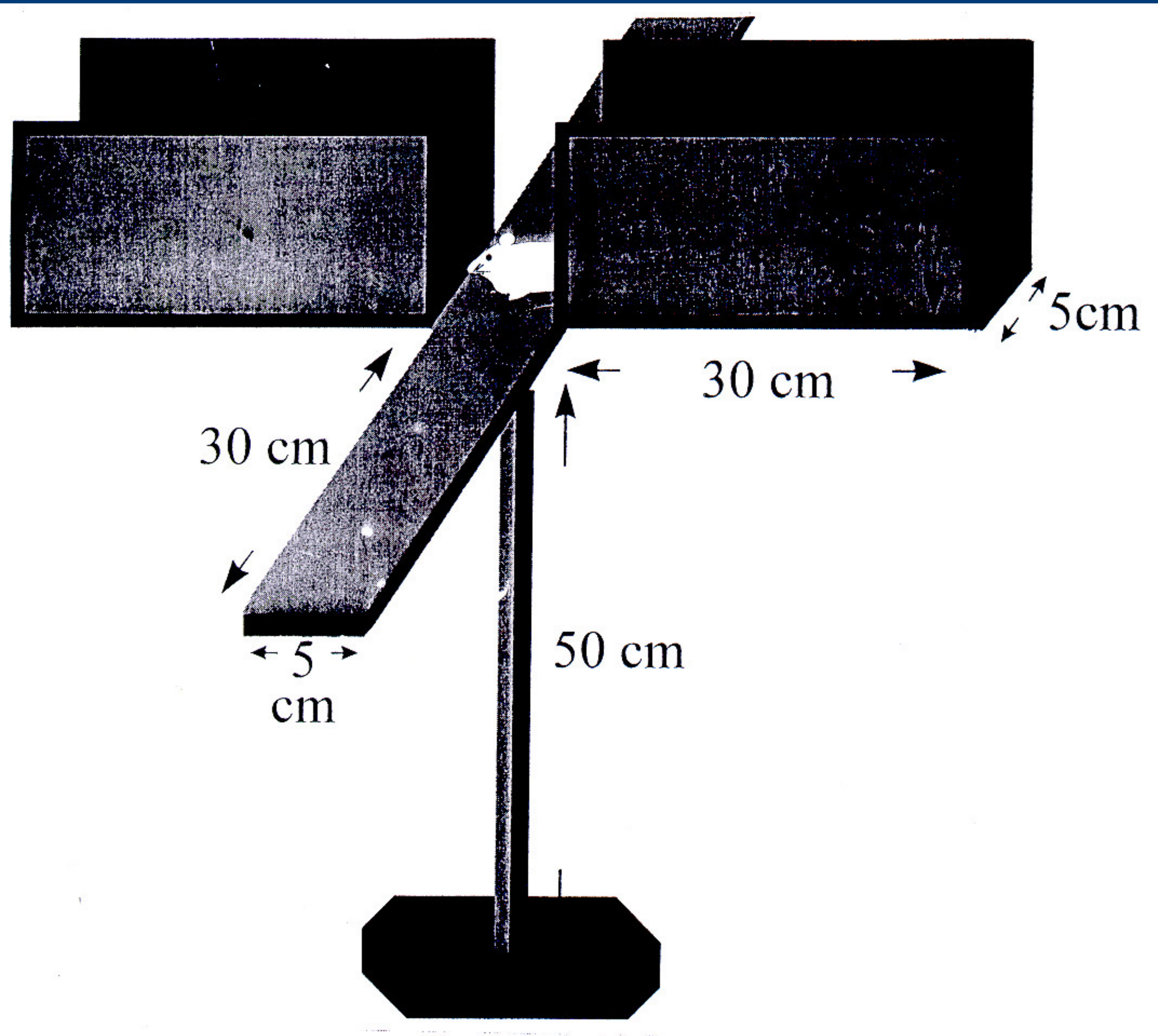
軌跡偵測儀





電腦




5cm
30 cm
30 cm
5
cm
50 cm

- 評估指標
  - 進入開放臂及封閉臂時間
  - 進入開放臂及封閉臂次數
- 焦慮與抗焦慮之評估標準

| 評估標準 | 焦慮 | 抗焦慮 |
|---|---|---|
| 進入開放臂時間和次數百分率 | ↓ | ↑ |
| 進入封閉臂時間和次數百分率 | ↑ | ↓ |

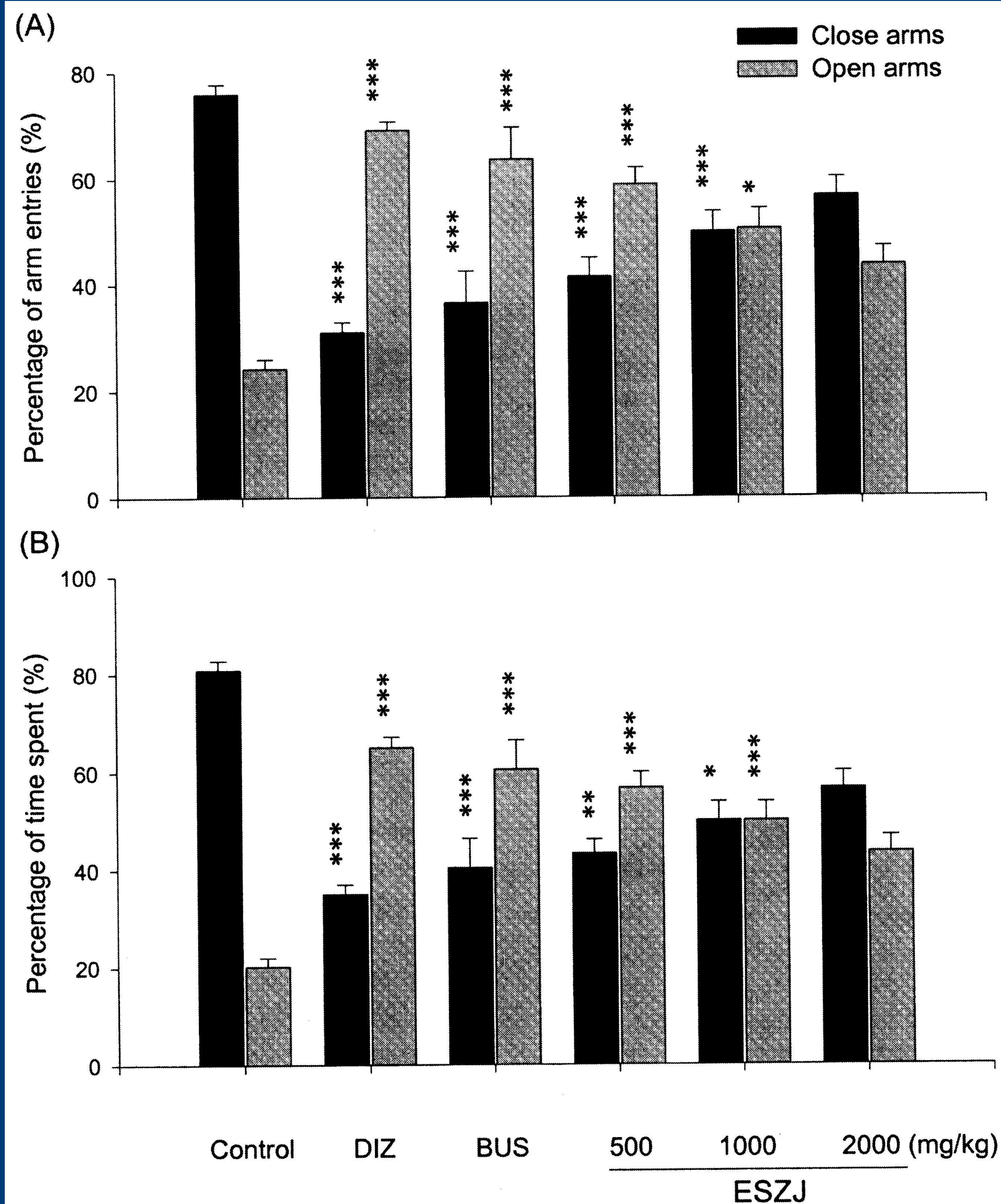



Fig. 4. Effects of diazepam (DIZ), buspirone (BUS) and the ethanolic extract of SZJ (ESZJ) on (A) the percentage of arm entries and (B) the percentage of time spent in the close arms and open arms of the elevated plus-maze during a 5-min test in mice. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ as compared with the control group (one-way ANOVA following by Scheffe test).

# 小結 1

1. 黑白室誘發焦慮模式：
   - 延長之小鼠在白室之滯留時間，
   - 減少在黑室之滯留時間，
2. 舉臂式十字形迷宮：
   - 增加小鼠在開放臂之滯留時間及進入次數百分率，
   - 減少小鼠在封閉臂之滯留時間及進入次數百分率，
3. 顯示酸棗仁乙醇萃取物具有抗焦慮作用。

# 小蘗鹼對於小鼠抗焦慮作用機轉之研究

Anxiolytic Effect of Berberine on Exploratory Activity of the Mouse in Two Experimentary Anxiety Models: Interaction with Drugs Acting at 5-HT Receptors

(Peng WH, Hsieh MT et al. Life Sci 75, 2451-2462, 2004)

1. 黑白室
2. 舉臂式十字迷宮

黃連 *Coptis chinensis* Franch.



藥材黃連—味連




藥材黃連—雲連




藥材黃連—雅連



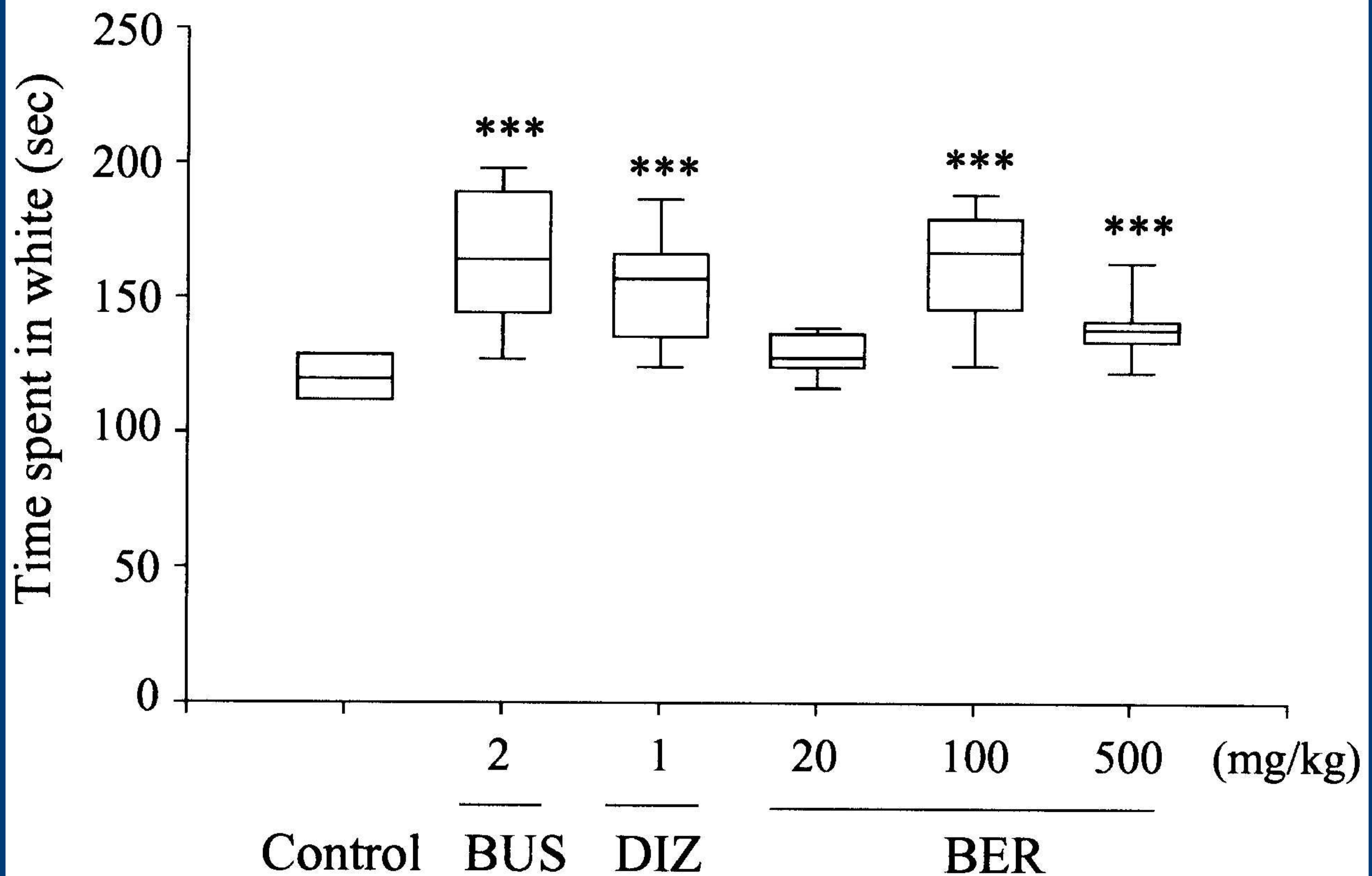



Fig. 2. Effects of berberine (BER), buspirone (BUS) and diazepam (DIZ) on the time spent in the black and white chambers of the black and white test in mice. Each column, center line in the column and the bars represent the 95% confidence interval, median and range of 12–18 mice, respectively. ***P < 0.001 as compared with the control group. (Kruskal-Wallis H following by Mann-Whitney *U*-test.

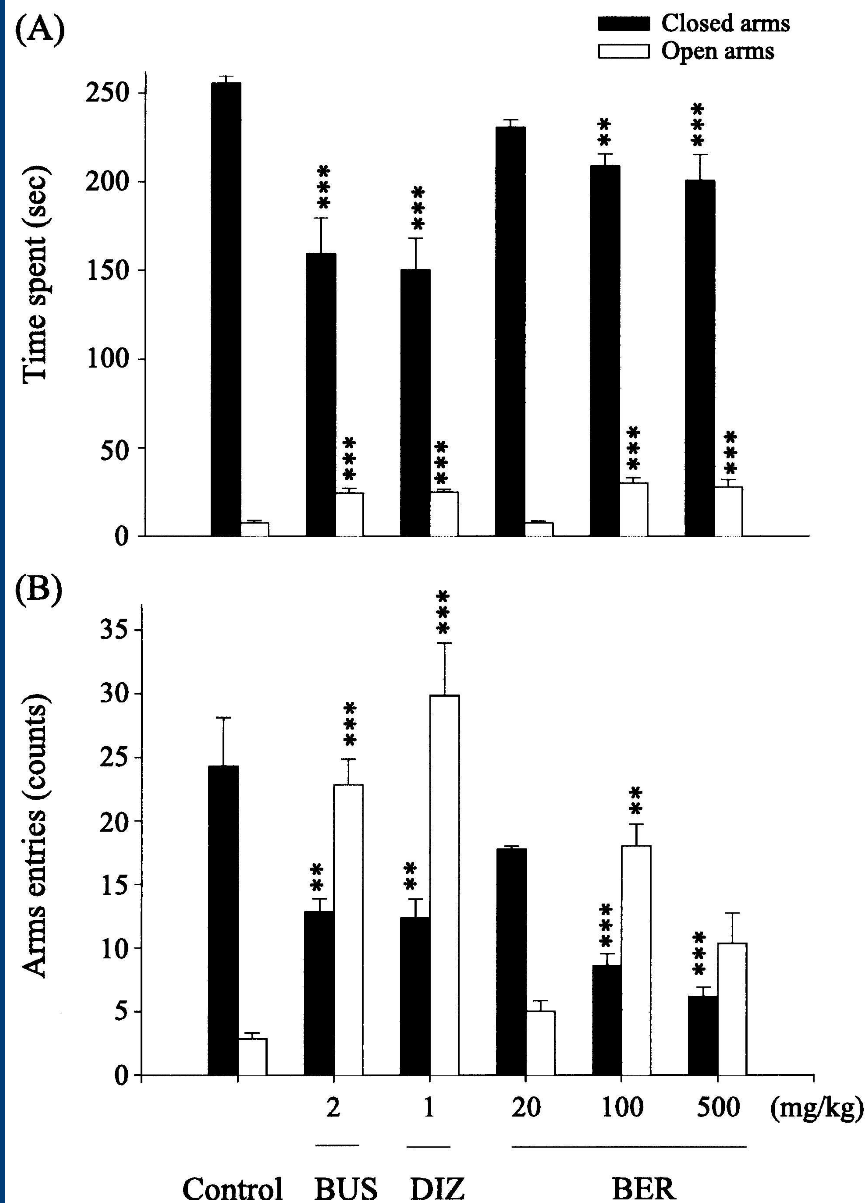



Fig. 4. Effect of berberine (BER), buspirone (BUS) and diazepam (DIZ) on (A) time spent (B) arm entries in the open arms and closed arms of the elevated plus-maze procedure in mice. Data are presented as mean±SEM, n=12–18. **$P < 0.01$, ***$P < 0.001$ compared with control group.

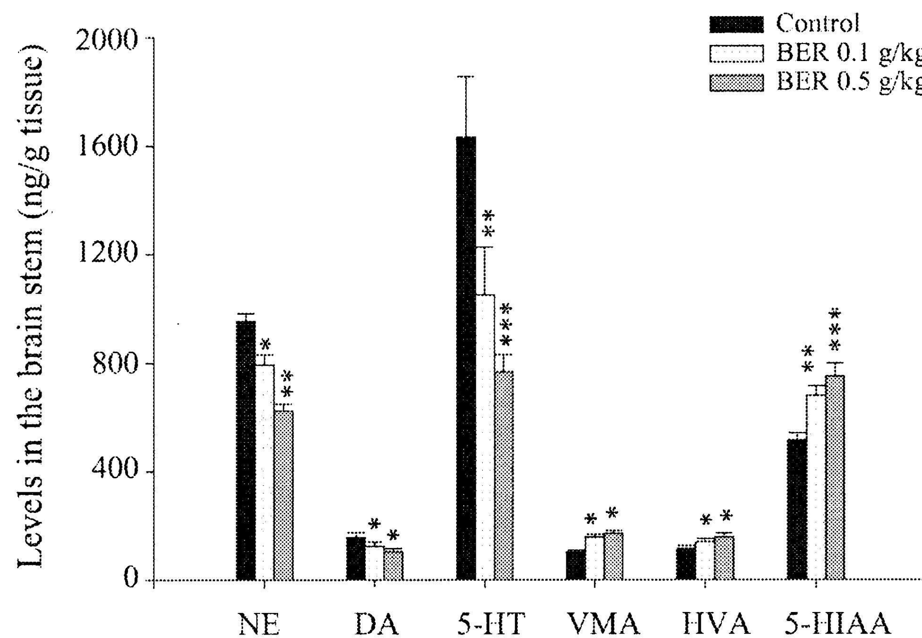



Fig. 6. Effects of BER on the levels of monoamine and its metabolites in the brain stem of mice. *P < 0.05, **P < 0.01, ***P < 0.001 as compared with the control group, respectively (One-way ANOVA followed by Scheffe's test).

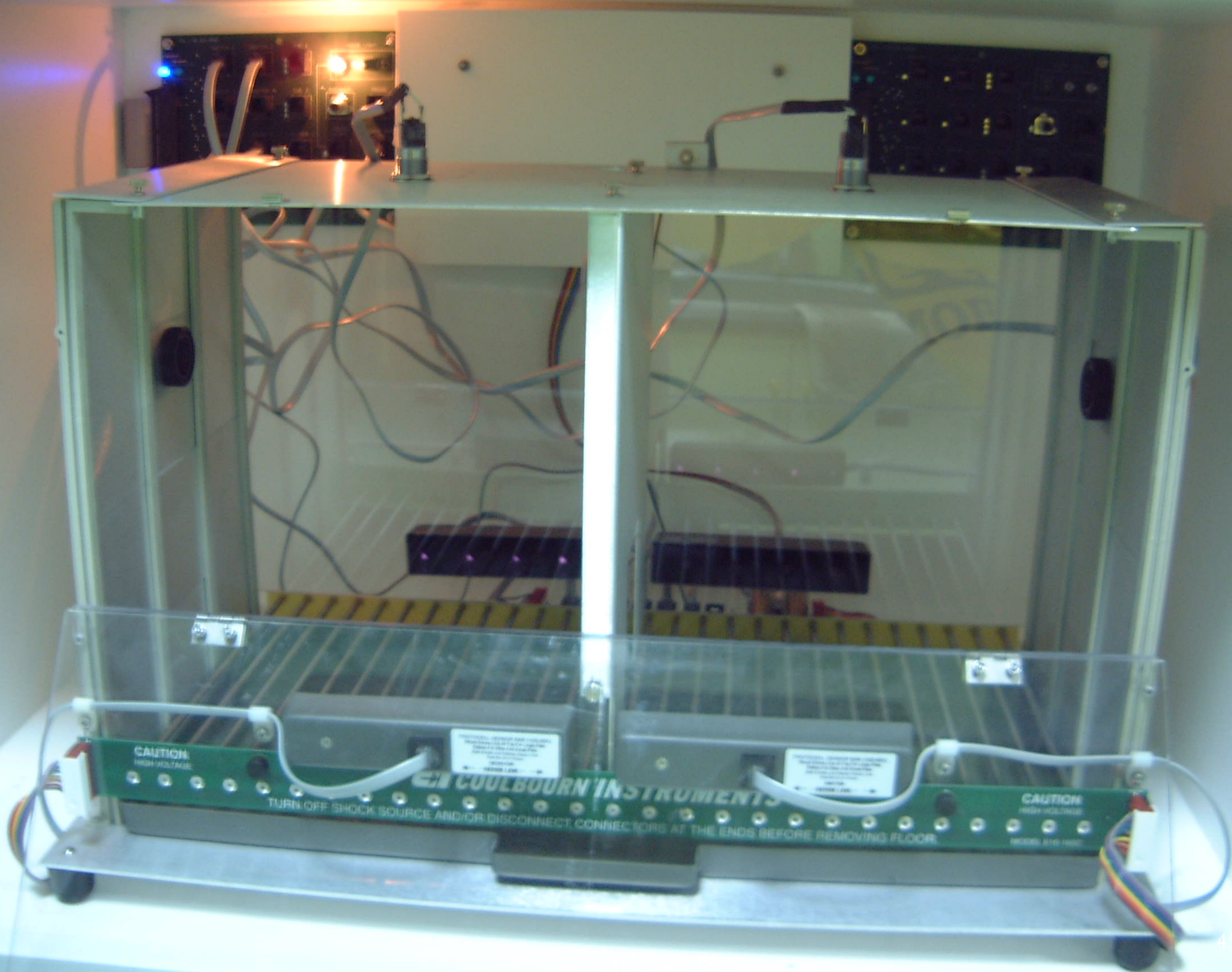
# 小結 2

- 黑白室誘發焦慮模式：
  - 延長之小鼠在白室之滯留時間，
  - 減少在黑室之滯留時間，
- 舉臂式十字形迷宮：
  - 增加小鼠在開放臂之滯留時間及進入次數，
  - 減少小鼠在封閉臂之滯留時間及進入次數，
- 顯示小蘗鹼(100 mg/kg, p.o.)具有抗焦慮作用。
- 小蘗鹼抗焦慮作用之機制主要與增加腦幹單胺轉化率及降低中樞serotonergic neuronal activities （特別是5-HT1A、5-HT2 receptors）有關

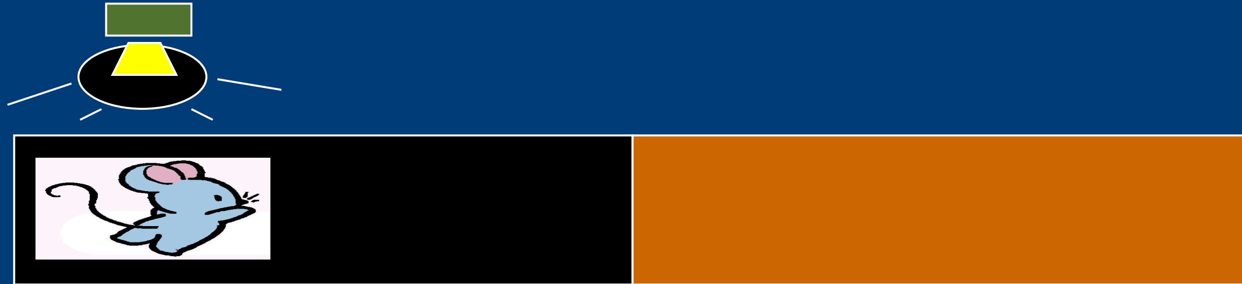
# 小蘗鹼增強大鼠記憶作用之研究

Effect of Long-term Administration of Berberine on Scopolamine-induced Amnesia in Rats

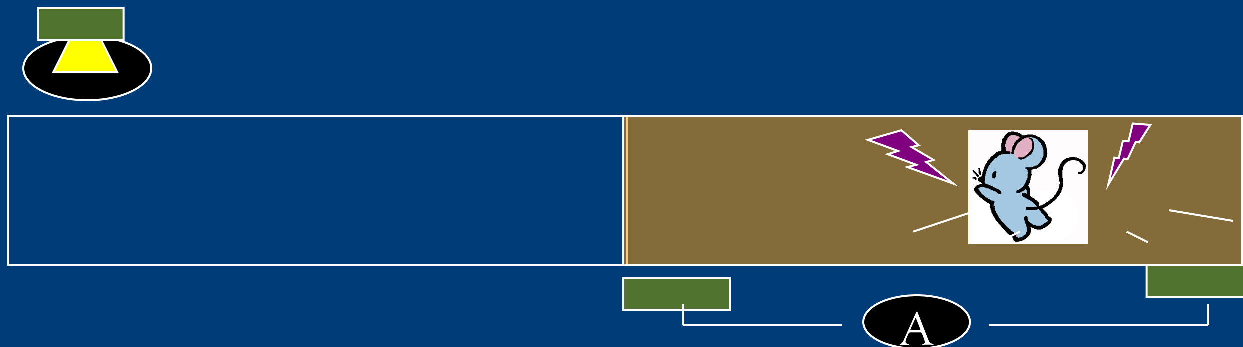
*(Peng WH, Hsieh MT et al. Jpn J Pharmacol 74, 261-266, 1997)*


CAUTION
HIGH VOLTAGE
COULBOURN INSTRUMENTS
TURN OFF SHOCK SOURCE AND/OR DISCONNECT CONNECTORS AT THE ENDS BEFORE REMOVING FLOOR.
CAUTION
HIGH VOLTAGE

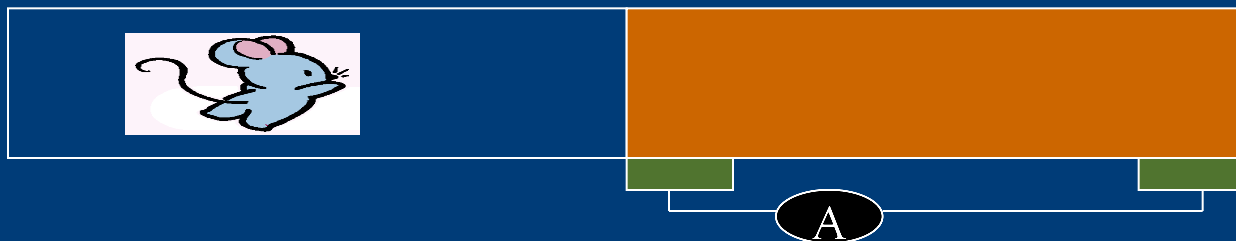
(1) 動物放入明室



(2) 關上閘門，通以電流 (1 mA, 2 sec)




(4) 24小時後，再將老鼠放入明室



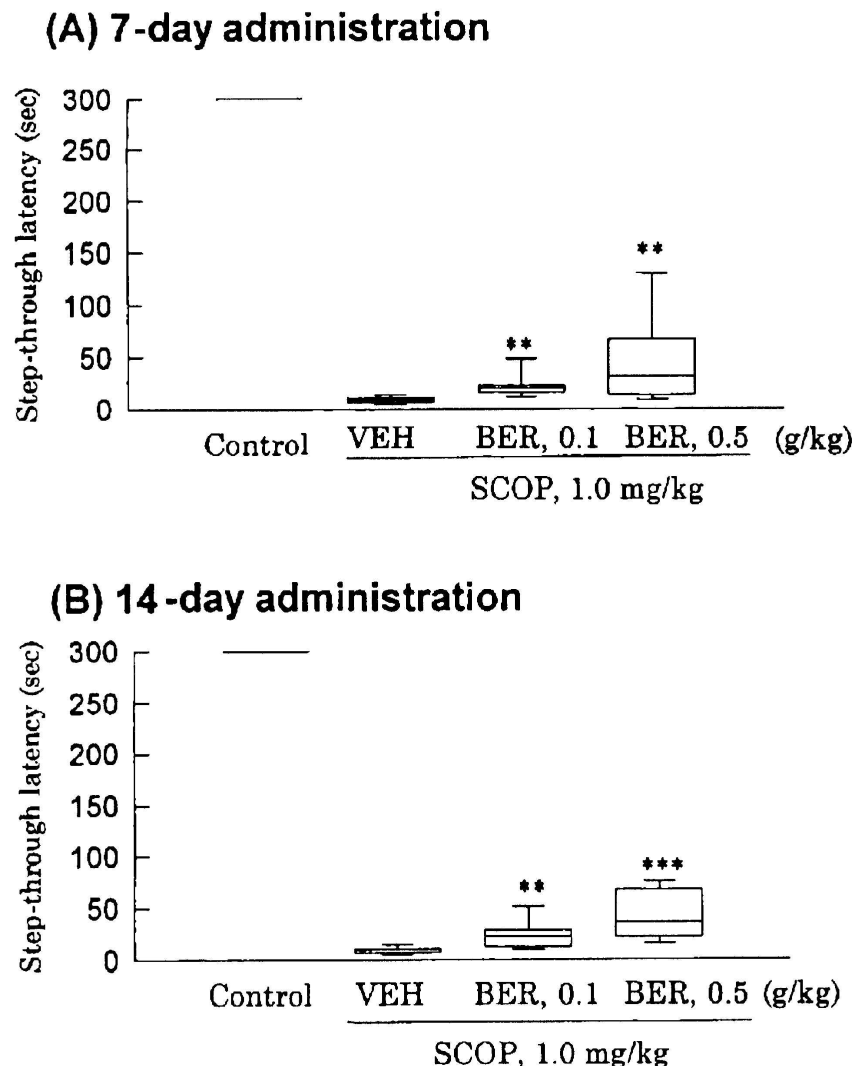



**Fig. 1.** Effects of berberine (BER) administered to scopolamine (SCOP)-treated rats on SCOP-induced amnesia in rats. A: BER was administered for 7 days B: BER was administered for 14 days. Each column, center line in the column and the bars represent the 95% confidence interval, mediam and range of 12−18 rats, respectively. $^{**}P<0.01$, $^{***}P<0.001$ compared with the SCOP group.

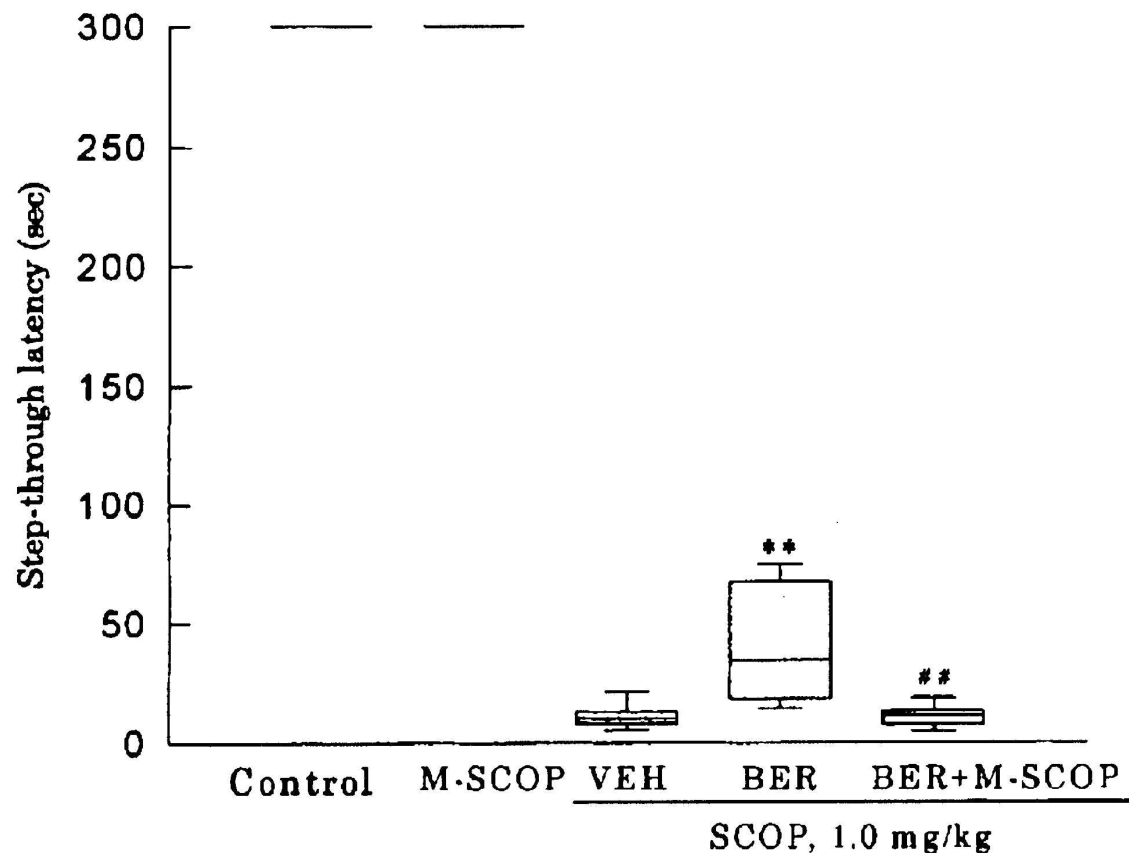



**Fig. 2.** Effects of scopolamine methylbromide (M-SCOP, 0.5 mg/kg) on berberine (BER, 0.5 g/kg)-induced recovery from SCOP-induced amnesia in rats. BER was administered for 14 days. Each column, center line in the column and the bars represent the 95% confidence interval, median and range of 12−18 rats, respectively. $^{**}P<0.01$, compared with the SCOP group. $^{\#\#}P<0.01$, compared with the group given SCOP in combination with BER.

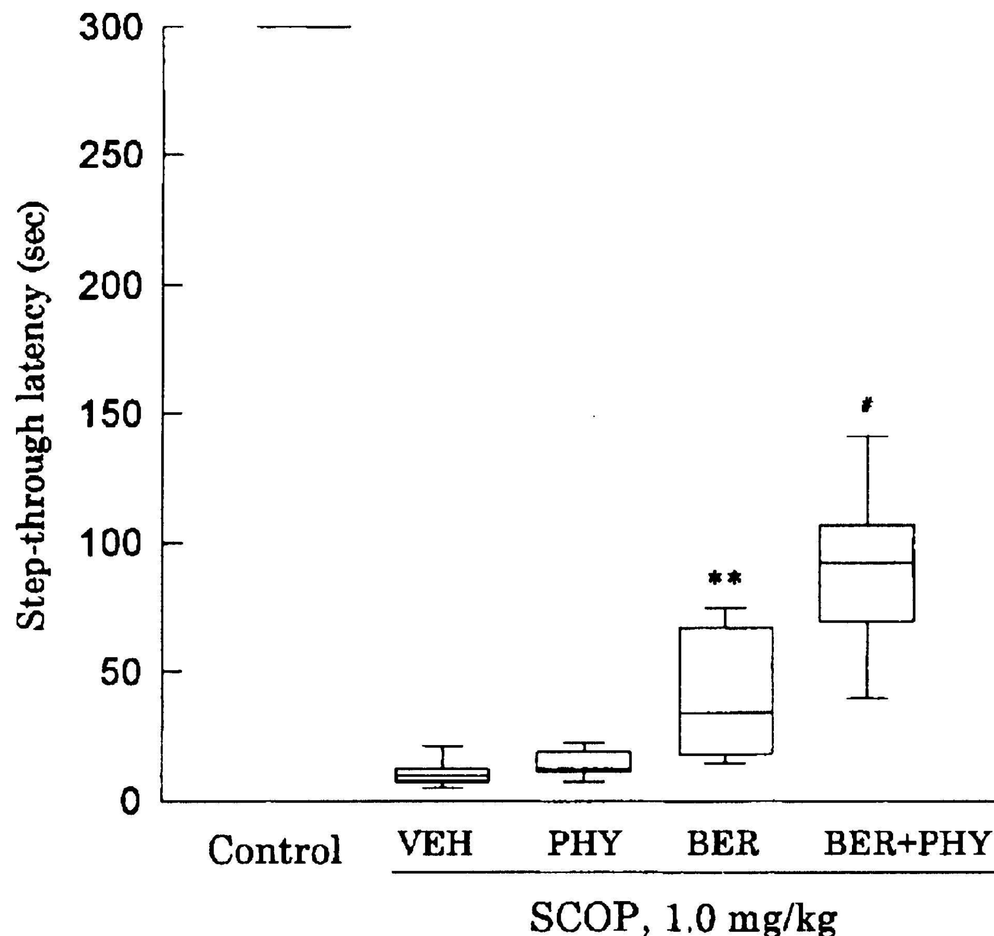



**Fig. 3.** Effects of physostigmine (PHY, 0.02 mg/kg) on berberine (BER, 0.5 g/kg)-induced recovery from scopolamine (SCOP)-induced amnesia in rats. BER was administered for 14 days. Each column, center line in the column and the bars represent the 95% confidence interval, median and range of 12−18 rats, respectively. **$P<0.01$, compared with the SCOP group. #$P<0.05$, compared with the group given SCOP in combination with BER.

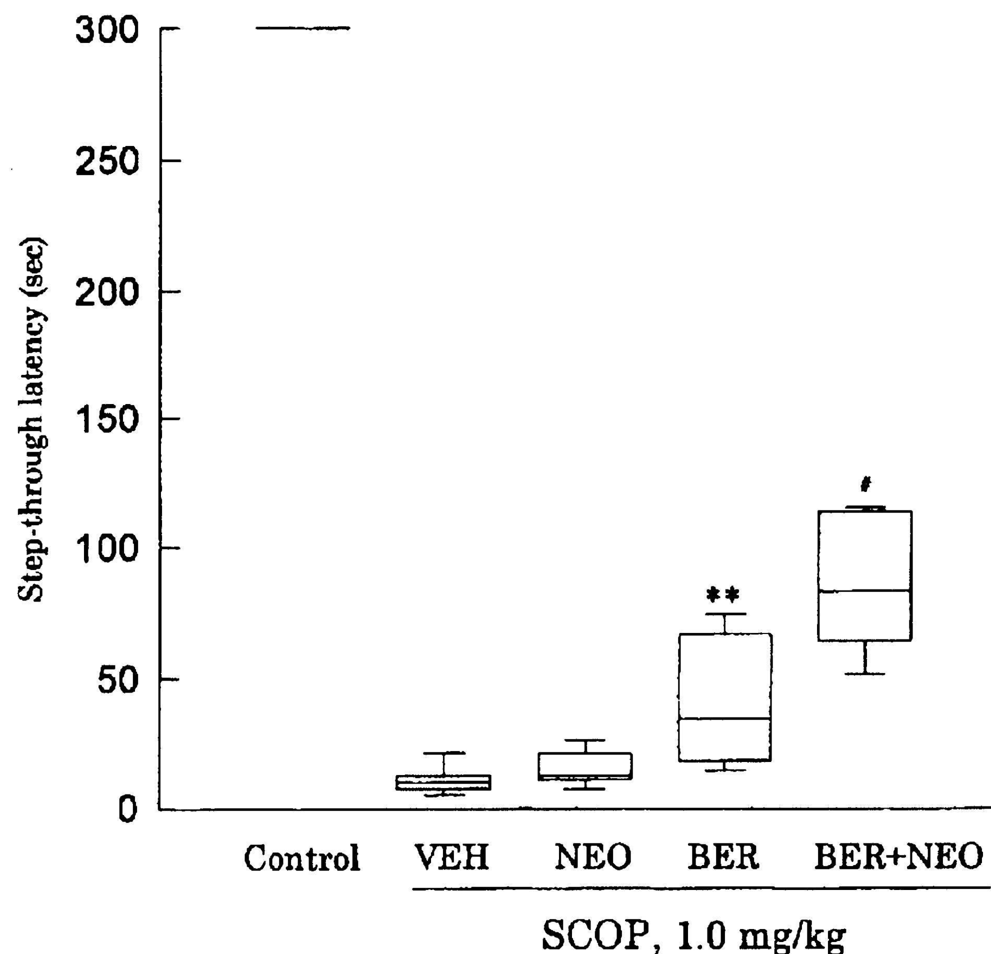



**Fig. 4.** Effects of neostigmine (NEO, 0.02 mg/kg) on berberine (BER, 0.5 g/kg)-induced recovery from scopolamine (SCOP)-induced amnesia in rats. BER was administered for 14 days. Each column, center line in the column and the bars represent the 95% confidence interval, median and range of 14 – 17 rats, respectively. **$P < 0.01$, compared with the SCOP group. $^{\#}P < 0.05$ compared with the group given SCOP in combination with BER.

# 小結 3

- 大鼠口服給予小蘗鹼7天及14天，可增強大鼠記憶能力，其作用機轉與增強末梢及中樞cholinergic neuronal system活性有關。

# 木犀草素改善類澱粉樣蛋白誘發大鼠學習記憶障礙及其對神經細胞保護作用之分子機制研究

*(Cheng HY, Hsieh MT et al., Phytotherapy Research, 2010;*
*Tsai FS, Hsieh MT et al., American Journal of Chinese Medicine, 2010)*

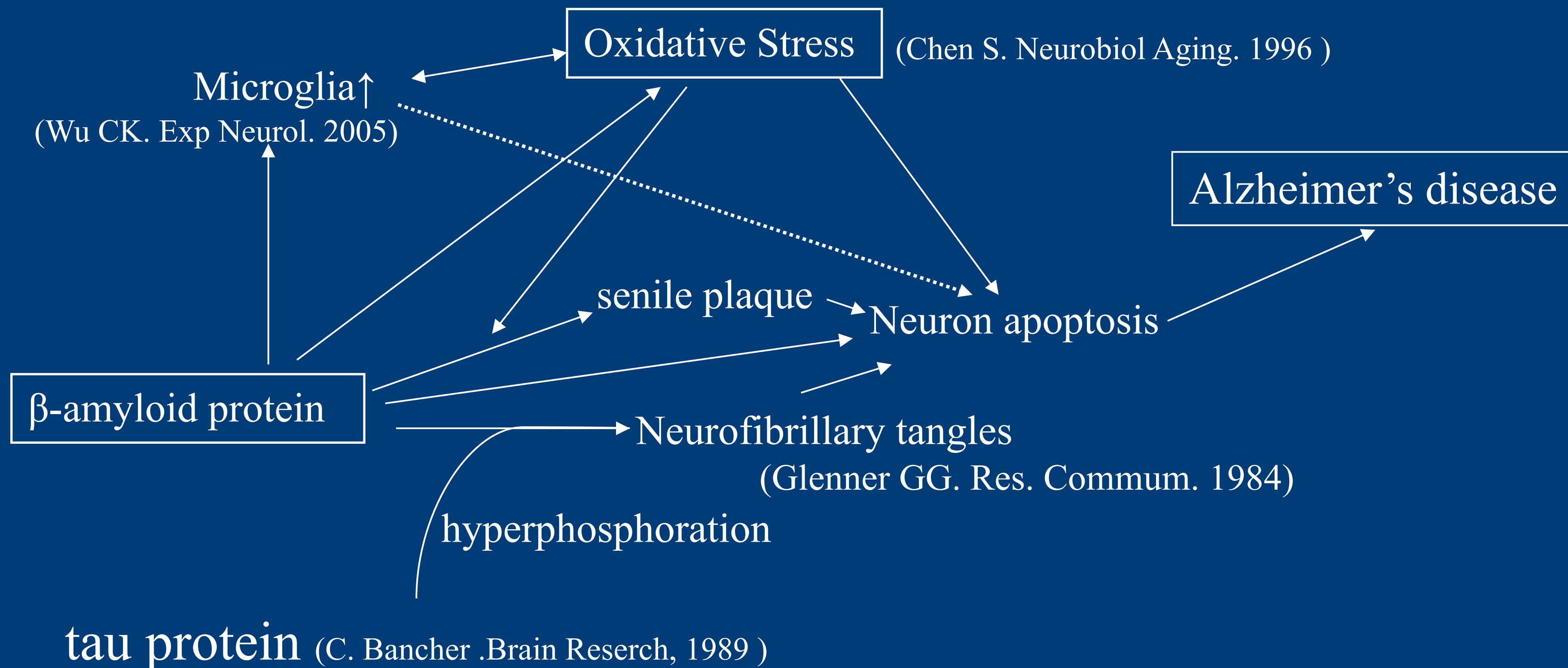
# 研究背景(一)

- 據主計處於民國95年國情統計台灣癡呆症病人約有10萬人。
- 癡呆症 (dementia)是一種引起腦神經系統退化的疾病 (Butterfield DA. *Trends Mol Med.* 2001.)
  - 臨床症狀為記憶力、定向力、判斷力、思考力、語言、行為、情緒及個性改變等症狀。
- 癡呆症之病因
  - 腦損傷、阿茲海默症 (Alzheimer’s disease)或巴金森氏症(Parkinson’s disease )

# 研究背景(二)

- 阿茲海默症約佔痴呆病人的60% (Eustache F. Rev Neurol. 2006.)
- 阿茲海默症病徵
  - 大腦皮質及海馬回中類澱粉樣蛋白過度分泌而大量沉積，導致神經元傷害(Glenner GG. Biophys Res Commun 1984)
    - 老人斑(Senile plaque)
    - 神經纏結(Neurofibrillary tangles)
    - 神經凋亡(neuronal apoptosis)

# 研究背景(三)
# Alzheimer’s disease mechanism



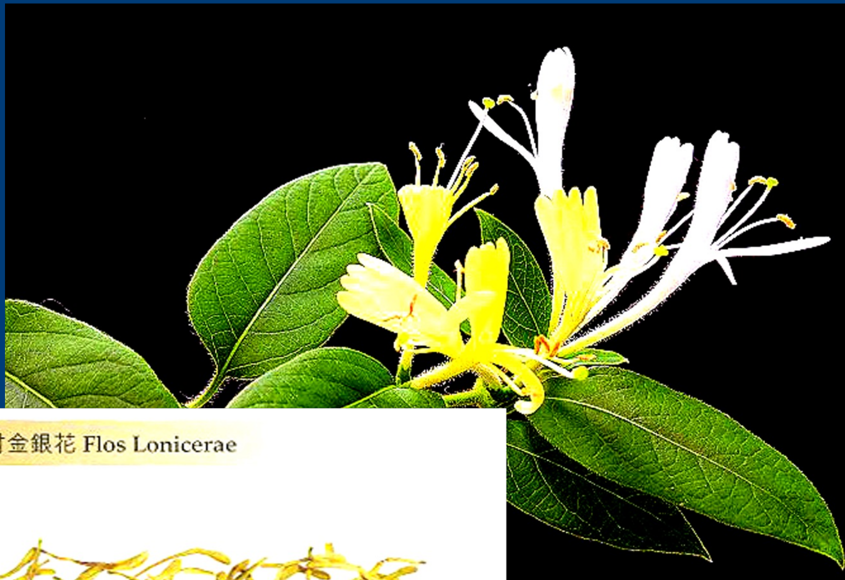
# 金銀花

- 忍冬科植物忍冬(*Lonicera japonica* Thunb.)的花蕾，又名金銀花。
- 花初開色白如銀，經數日變為黃色如金，新舊花相參，如金如銀，故名金銀花。
- 功能主治：清熱解毒。主治溫病發熱。
- 別錄：主寒熱身腫，久服輕身，長年益壽。
- 成分：木犀草素(Luteolin) 。

# 金銀花

## (*Lonicera japonica* Thunb.)

(當代藥用植物典第2冊，2006；pp51-5)

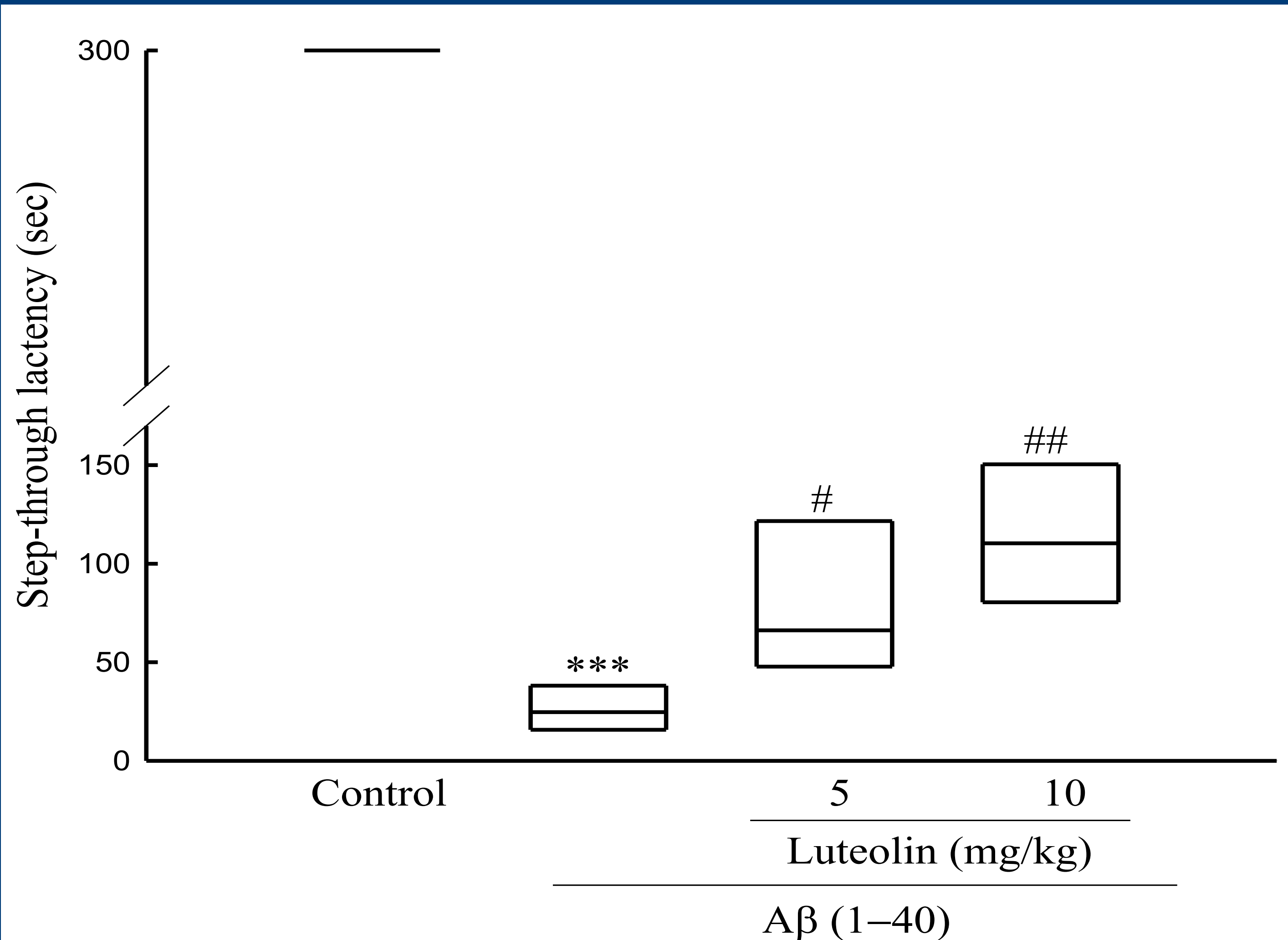




# 研究方法

- 在大鼠雙側巨大基底核 (NBM) 單次輸注類澱粉樣蛋白誘發大鼠學習操作障礙，可作為擬似阿茲海默症之動物模式(Giovannelli L. Neuroscience. 1995.)

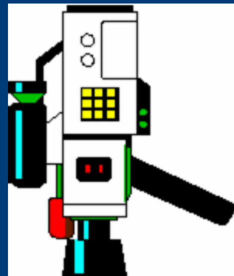
# 木犀草素對Aβ (1-40)誘發大鼠被動迴避之影響



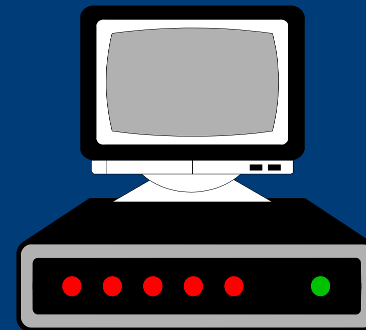
# 水迷宮結構圖

(Morris RG. Nature. 1982)

攝影機



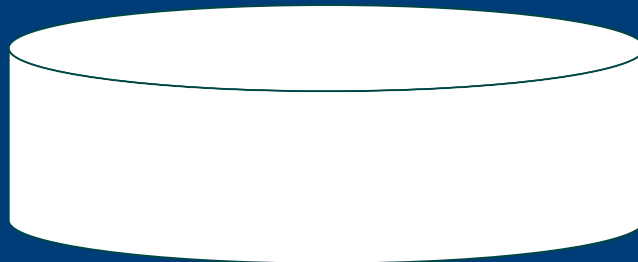


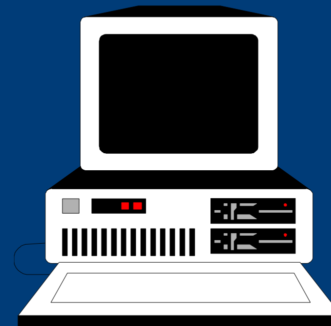


軌跡偵測儀



水池





電腦



Water Maze Version 3.20



**Information:**

<Esc> Return to Main Menu
<F1> HELP (toggle)
<F2> Toggle Analysis Results
<F3> Print all trial Maps from a loaded experiment
<F4> Make analyzed data files
<F5> Artifact Filter: [OFF]

Hit <RETURN> on an empty path to display experiments (*.exp)

Select experiment, then hit the <RETURN> key to show trials.

Select trial, <RETURN> to load

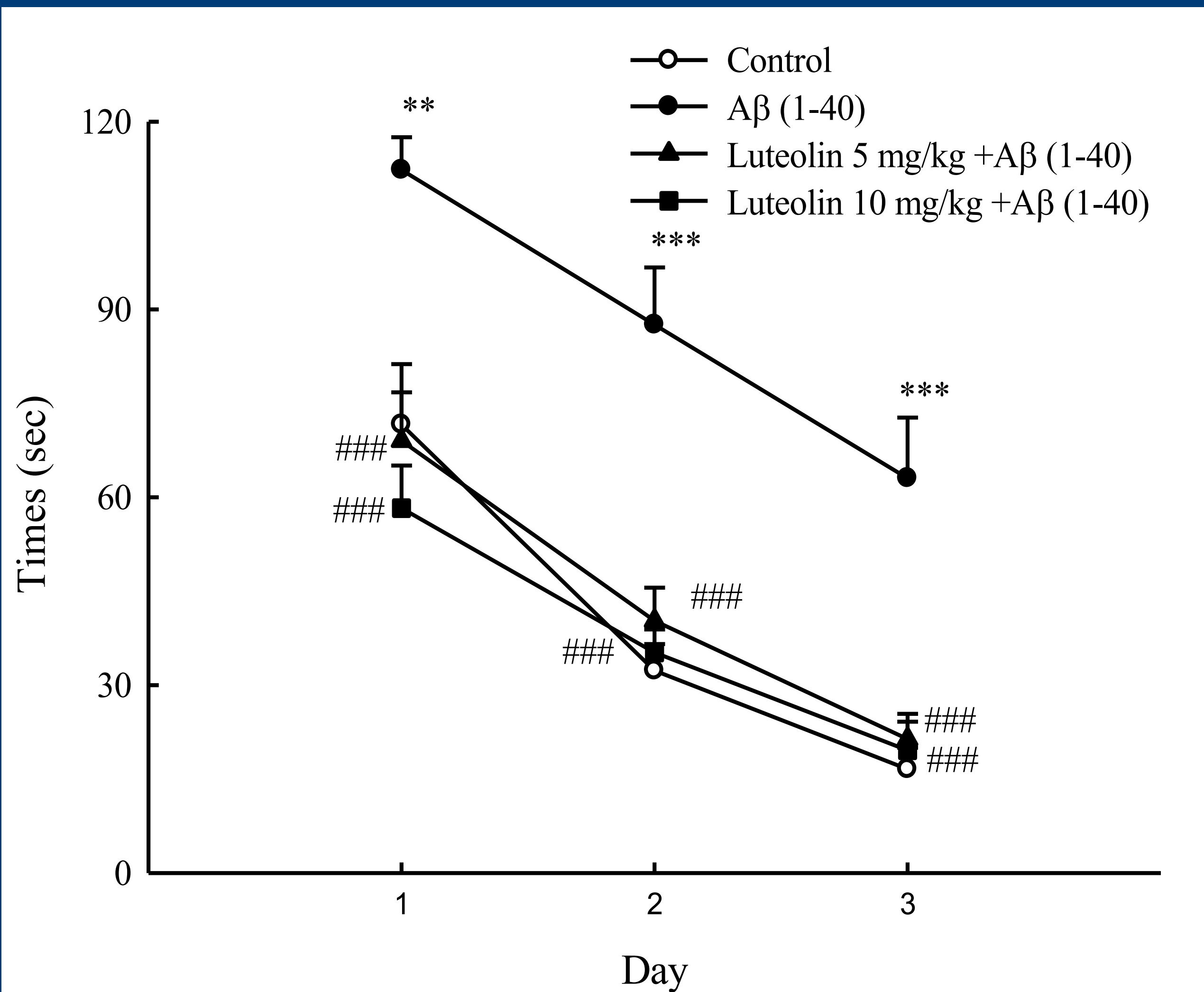
**RELOADING RAW DATA**

PATH\EXPERIMENT= d:\lez\1207\1207.EXP

Listing Trials: Trial= cat0111 File= 120716.RAW

| | | | | |
|---|---|---|---|---|
| 00. veh11 | 01. veh12 | 02. veh13 | 03. veh14 | 04. veh21 |
| 05. veh22 | 06. veh23 | 07. veh24 | 08. veh31 | 09. veh32 |
| 10. veh33 | 11. veh34 | 12. veh41 | 13. veh42 | 14. veh43 |
| 15. veh44 | 16.[cat0111 ] | 17. cat0112 | 18. cat0113 | 19. cat0114 |
| 20. cat0121 | 21. cat0122 | 22. cat0123 | 23. cat0124 | 24. cat0131 |

# 木犀草素對Aβ(1-40)誘發水迷宮空間性操作障礙之影響



# 小結 4

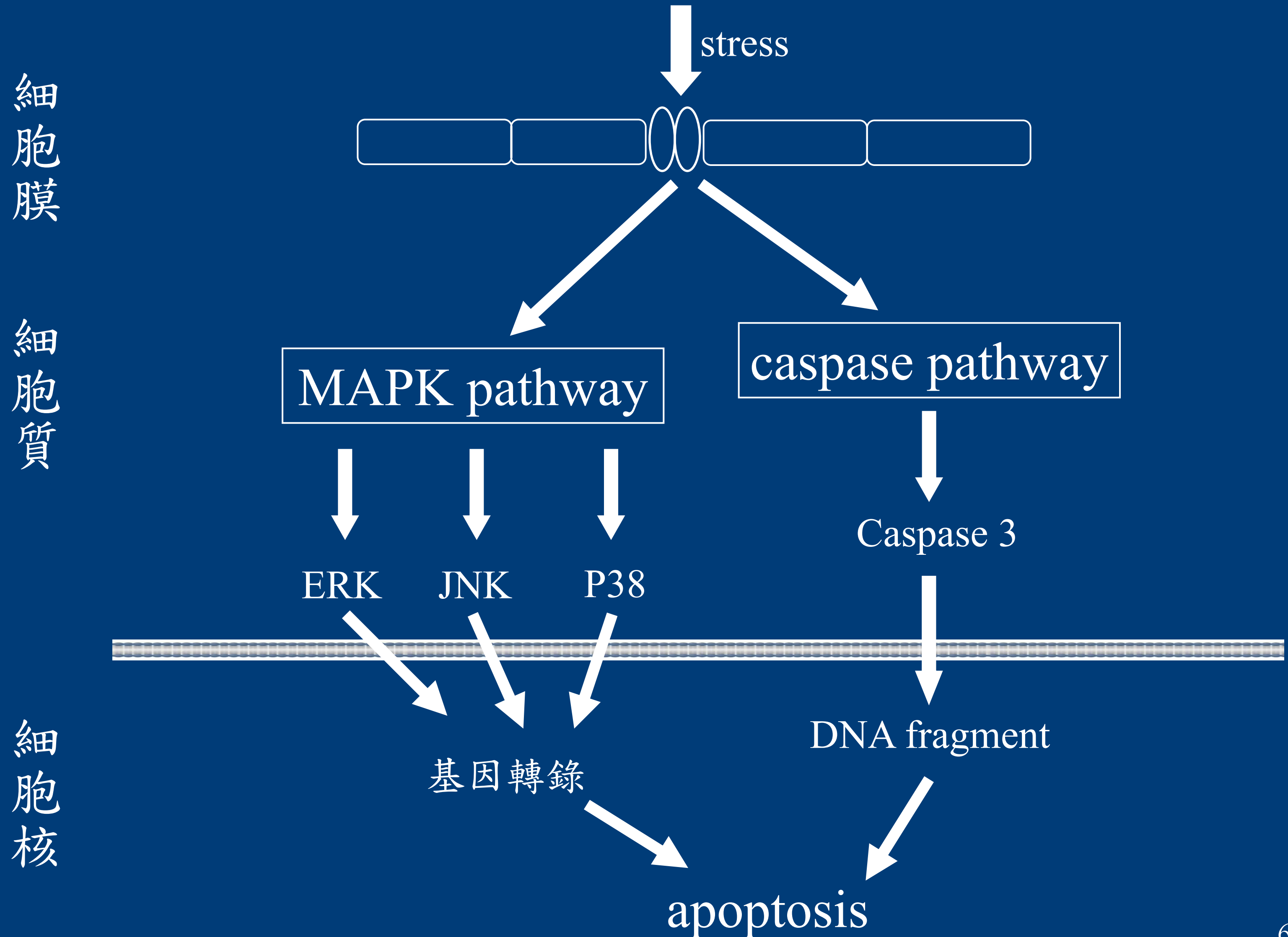
- 類澱粉樣蛋白
  - 造成被動迴避障礙
  - 水迷宮學習操作、工作及參考記憶能力之障礙
- 木犀草素
  - 改善被動迴避障礙
  - 改善學習操作、參考記憶、工作記憶之再學習及記憶再現障礙

# 木犀草素對類澱粉樣蛋白誘發細胞凋亡之分子訊息機制

# 細胞凋亡之分子訊息

- Protein kinase
  - Ras-MAPK pathway
    - JNK (Wanli W. et.al. Brain 2002)
    - ERK (Kuperstein F Eur J Neurosci 2002 )
    - P38 (Tamagno E Exp Neurol 2003 )
  - Caspases system (Wanli W. et.al. Brain 2002)
    - Caspase 3

# 細胞凋亡之分子訊息傳遞路徑



# 木犀草素改善Aβ(25-35)誘發細胞凋亡之分子機制探討

- Western blotting (Wanli W.,et. Brain. 2002.)
  - Protein kinase
    - JNK
    - ERK
    - P38
  - Caspases
    - Caspase 3

# 類澱粉樣蛋白片段作用之文獻考察

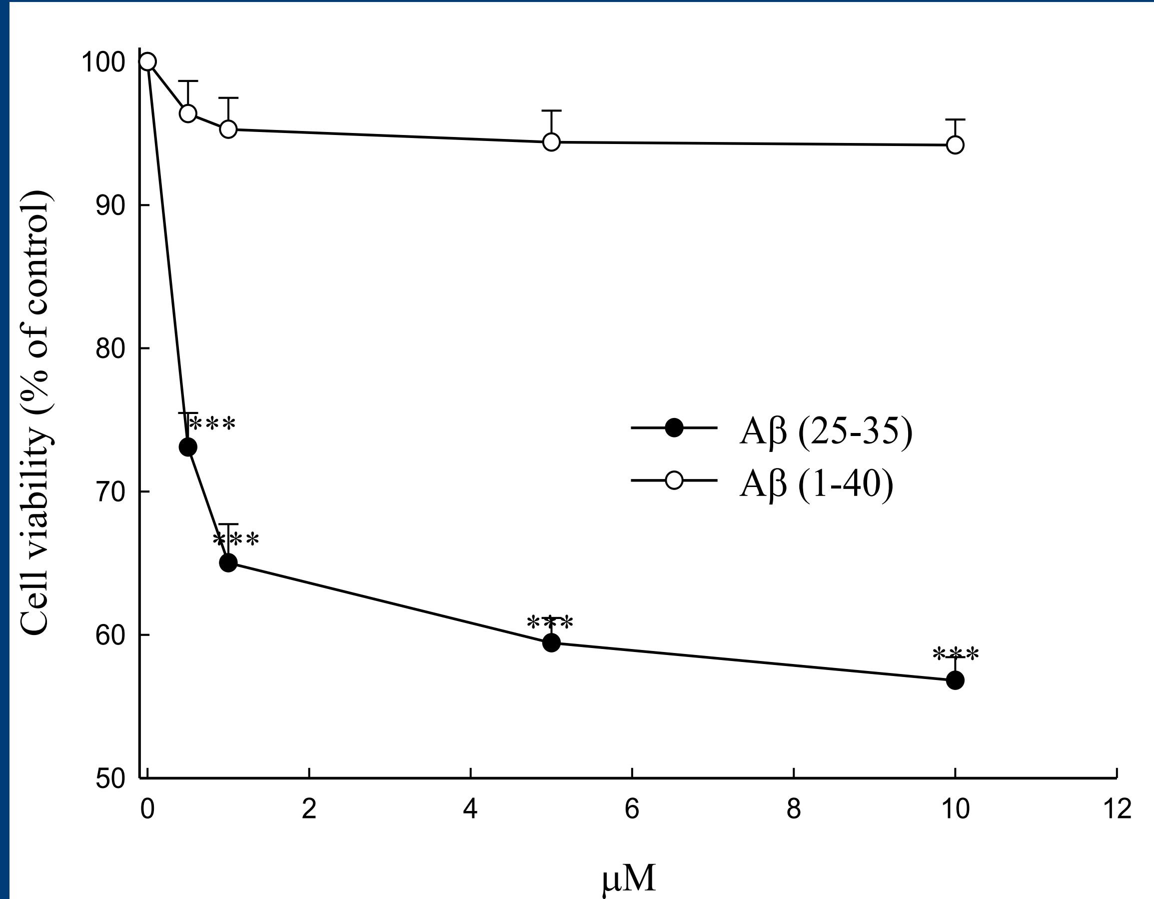
- Aβ(1-40)
  - 較接近屬於一般Aβ片段之生理作用，大部分文獻都使用於動物模式。
- Aβ(25-35)
  - 為完整類類澱粉樣蛋白造成神經毒性片段部分。

(Pike CJ. J Neurosci. 1993.)

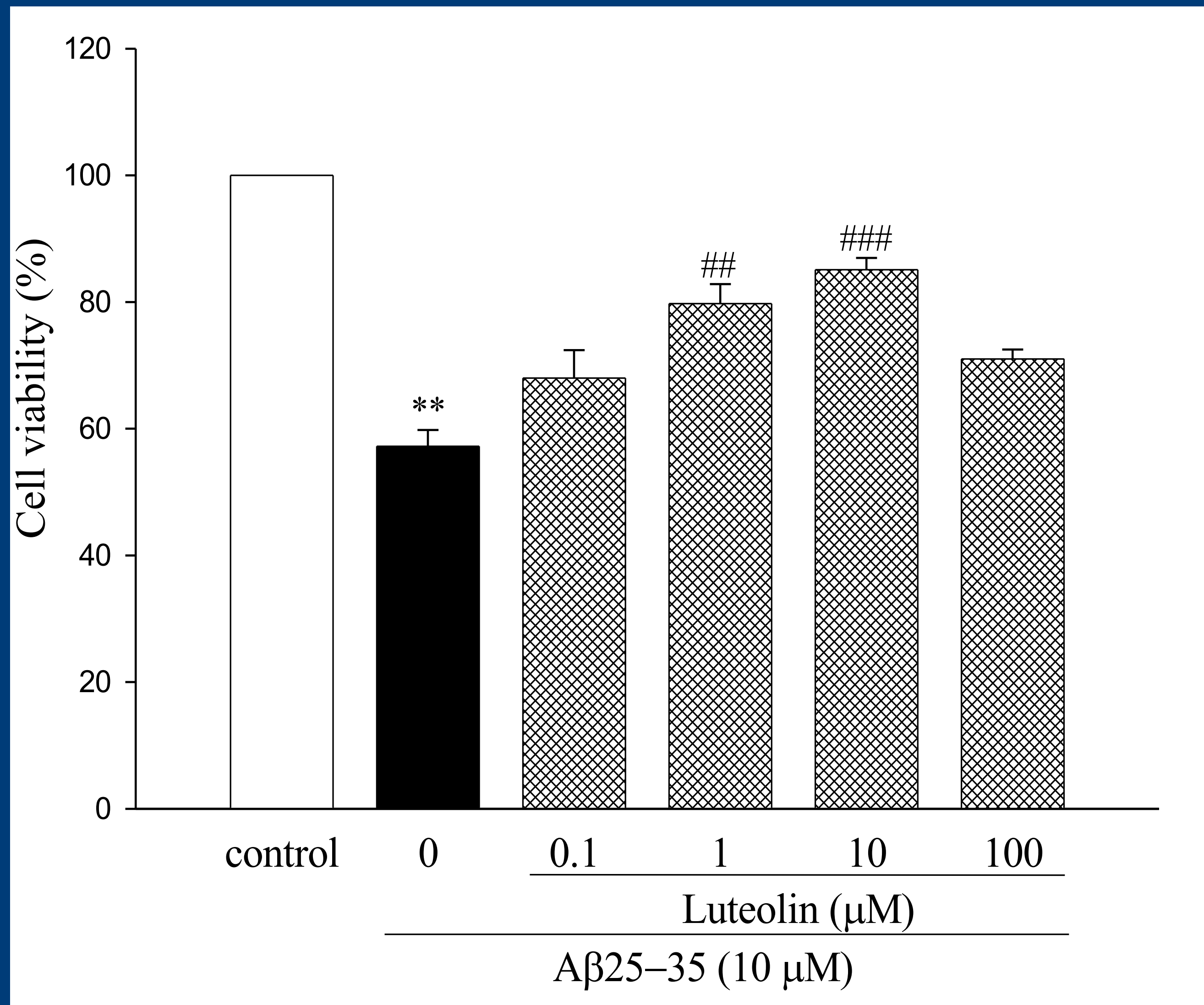
# 木犀草素對Aβ(25-35)誘發大鼠皮質細胞凋亡之影響

- Cell survive
  - MTT (Mandel S. Neurotox Res 2003.)
- DNA fragment (Sandstrom PA. Proc Natl Acad Sci USA 1993.)
- DAPI (Nardi N. J Neurochem 1997.)

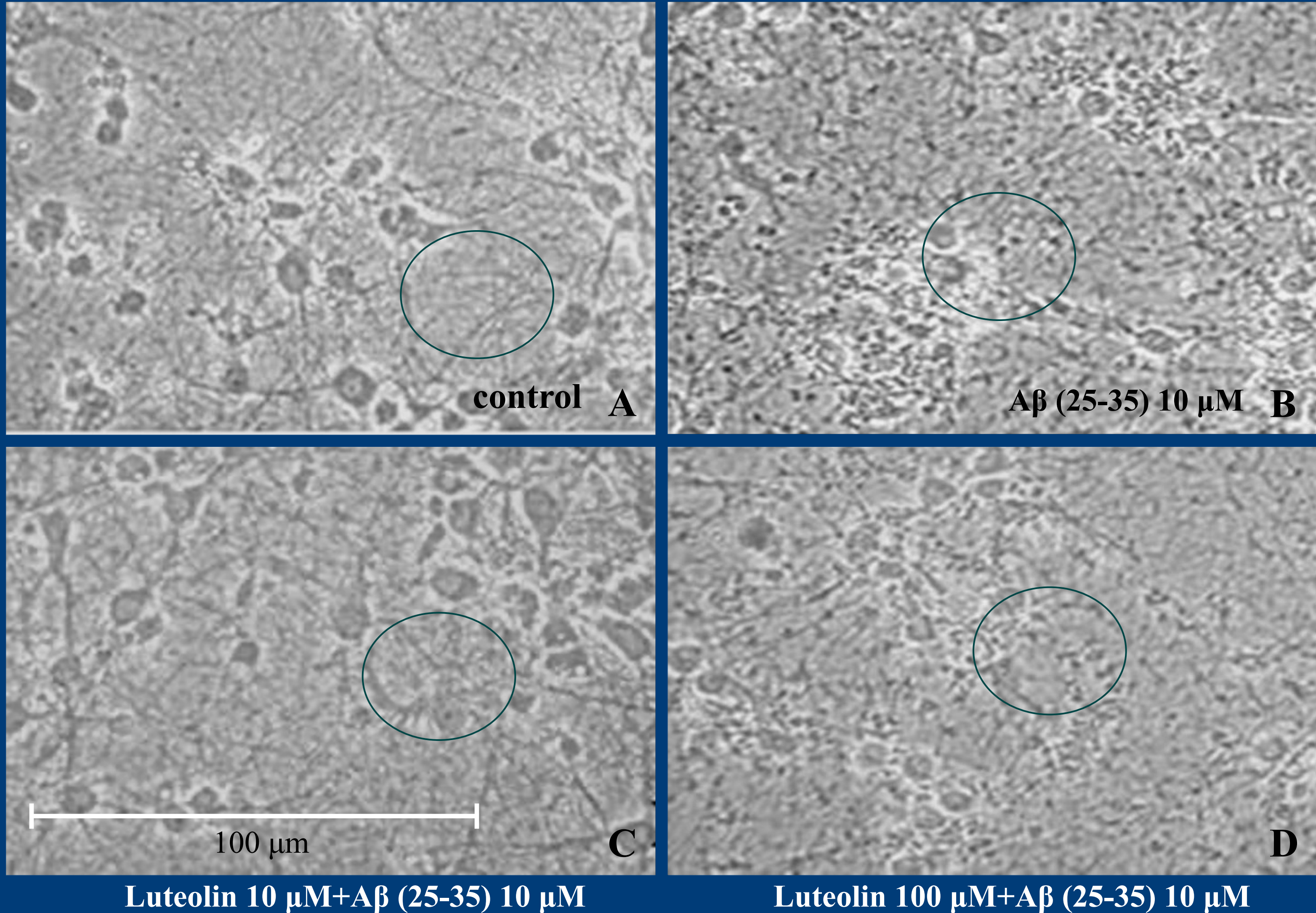
# Aβ (1-40) 、Aβ (25-35)對大鼠初代培養皮質神經細胞存活率之影響



# 木犀草素對Aβ (25-35)誘發大鼠初代培養皮質神經細胞存活率之影響



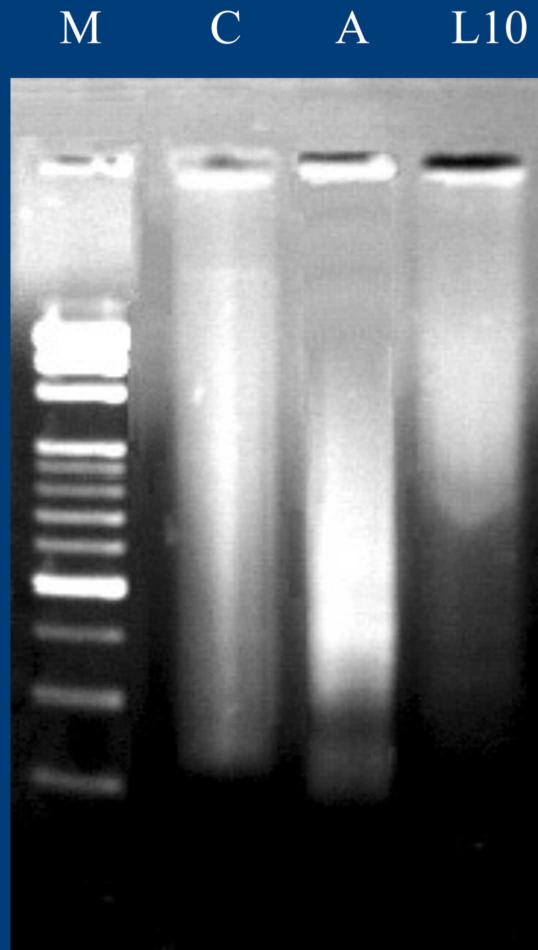
# 木犀草素對Aβ (25-35)誘發大鼠初代培養皮質神經細胞凋亡之細胞型態




Luteolin 10 μM+Aβ (25-35) 10 μM

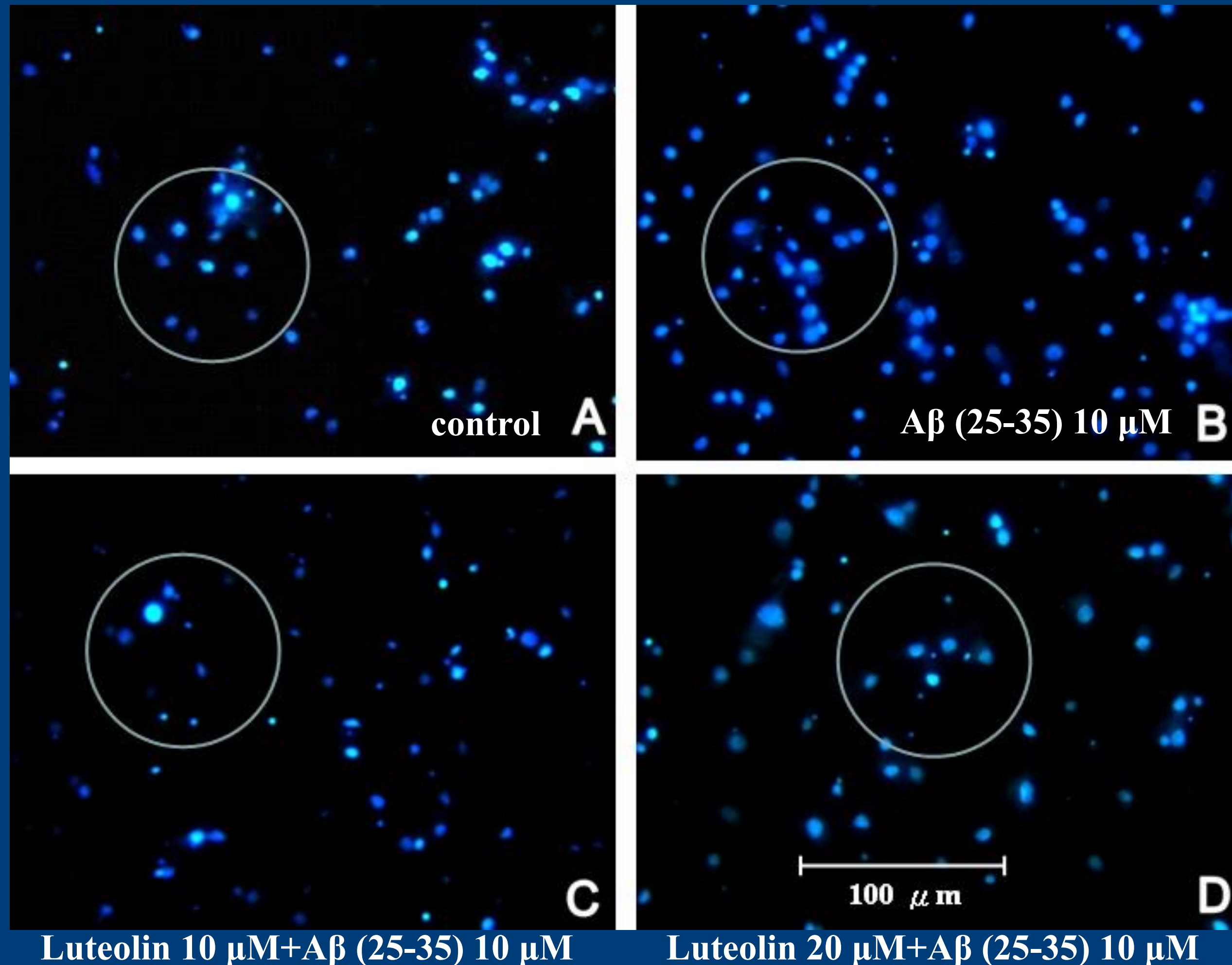
Luteolin 100 μM+Aβ (25-35) 10 μM

# 木犀草素對Aβ (25-35)誘發大鼠初代培養皮質細胞DNA斷片分析（DNA fragmentation assay）




M: marker
C: control
A: Aβ (25-35) 10 μM
L10: Luteolin 10 μM+ Aβ (25-35) 10 μM

# 木犀草素對Aβ (25-35)誘發大鼠初代培養皮質細胞凋亡之螢光染色（Apoptotic cells by DAPI staining）

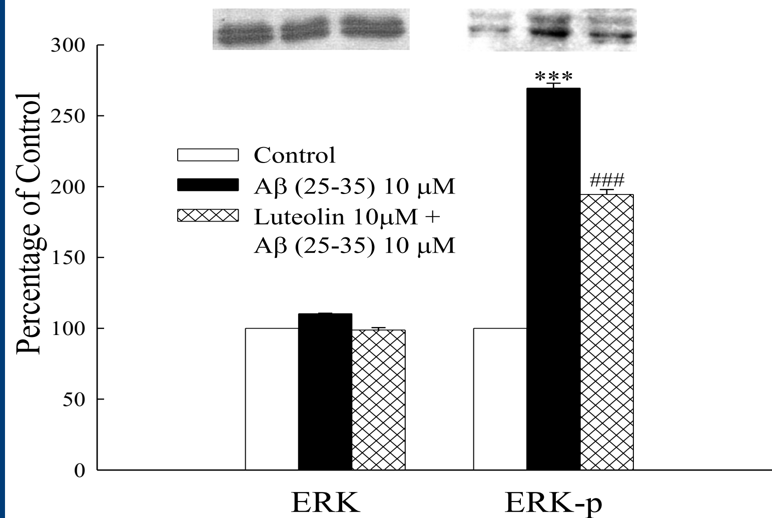


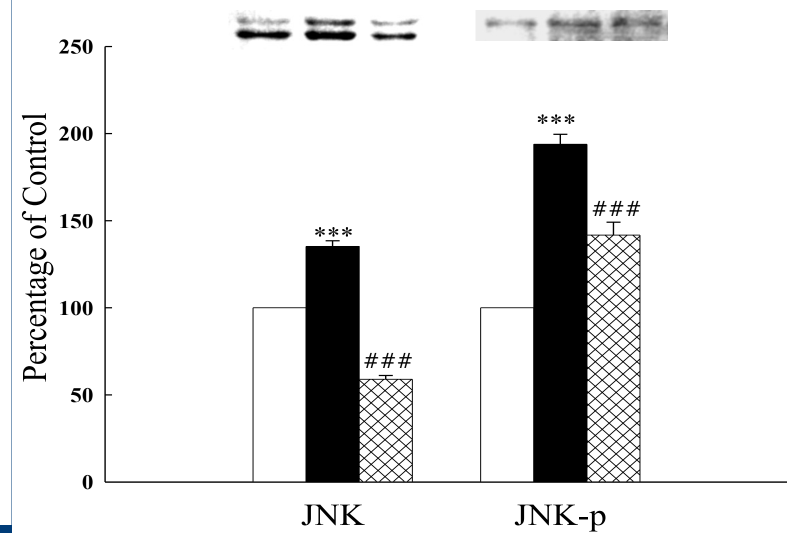

Luteolin 10 μM+Aβ (25-35) 10 μM    Luteolin 20 μM+Aβ (25-35) 10 μM

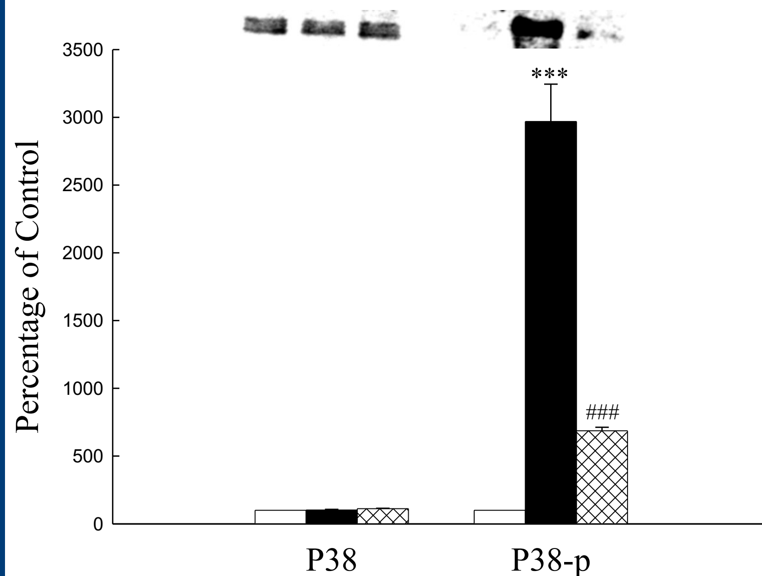
# 小結 5

- 類澱粉樣蛋白(25-35)
  - 具dose-dependent的細胞毒殺作用
  - 可經由光學顯微鏡觀察細胞型態、DAPI染色及DNA fragment等發現細胞處於凋亡狀態下
- 木犀草素
  - 經由上述結果發現，具有減輕細胞凋亡的作用

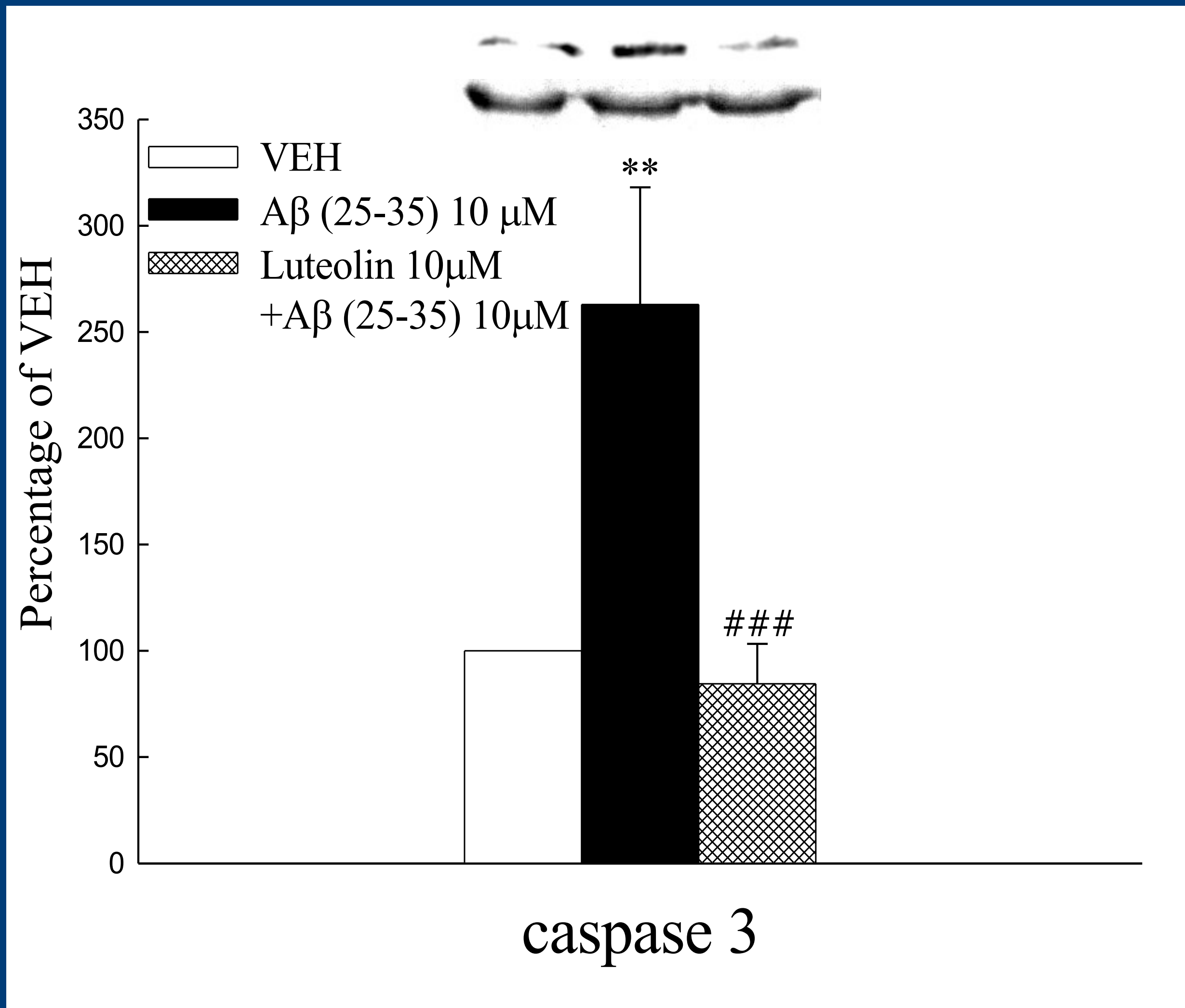
# 木犀草素保護類粉樣蛋白誘發大鼠皮質細胞凋亡與MAPK路徑之關係



# 木犀草素保護類粉樣蛋白誘發大鼠皮質細胞凋亡與caspase 3之關係



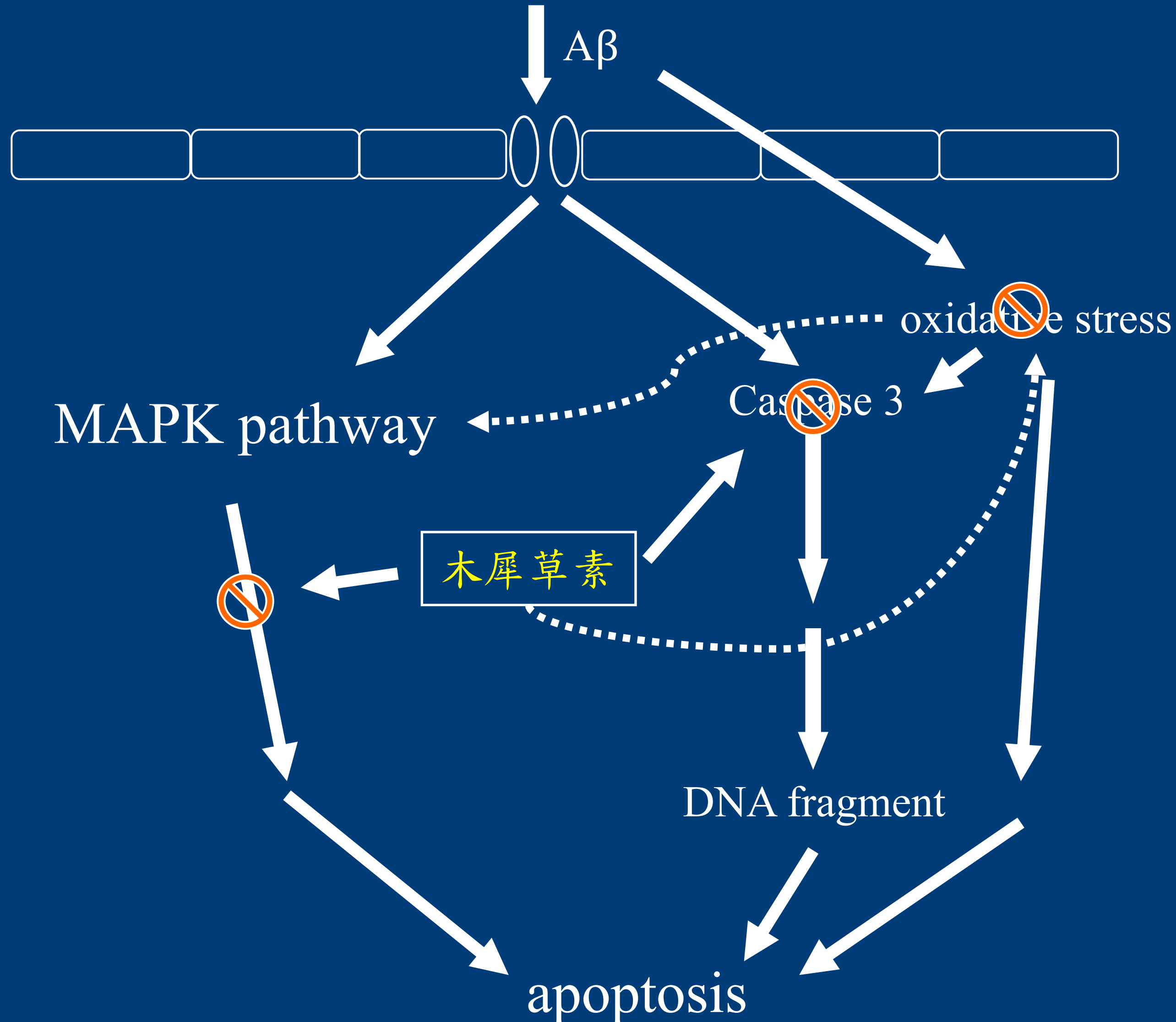
# 木犀草素對Aβ(25-35)誘發大鼠皮質細胞凋亡之影響

| | Aß (25-35) | Luteolin (10 μM) |
|---|---|---|
| ERK | - | - |
| ERK-p | ↑ | ↓ |
| JNK | ↑ | ↓ |
| JNK-p | ↑ | ↓ |
| P38 | - | - |
| P38-p | ↑ | ↓ |
| Caspase 3 | ↑ | ↓ |

# 小結 6

- 類澱粉樣蛋白
  - 經由活化ERK-p、JNK、JNK-p、P38-p、caspase 3來造成細胞凋亡的現象
- 木犀草素
  - 經由降低ERK-p、JNK、JNK-p、P38-p、caspase 3活化來達到保護的現象
  - 可改善類澱粉樣蛋白誘發之學習記憶障礙。
  - 其分子機制為抑制MAPK與caspase 3的活化。

# 木犀草素對於改善Aβ誘發學習記憶障礙之分子機制



# Scopolamine 對大鼠海馬迴(Hippocampus)基因表現之影響

- Hippocampus
  - 涉及刺激與學習之間的連結

(involving associations between stimulus and learning)

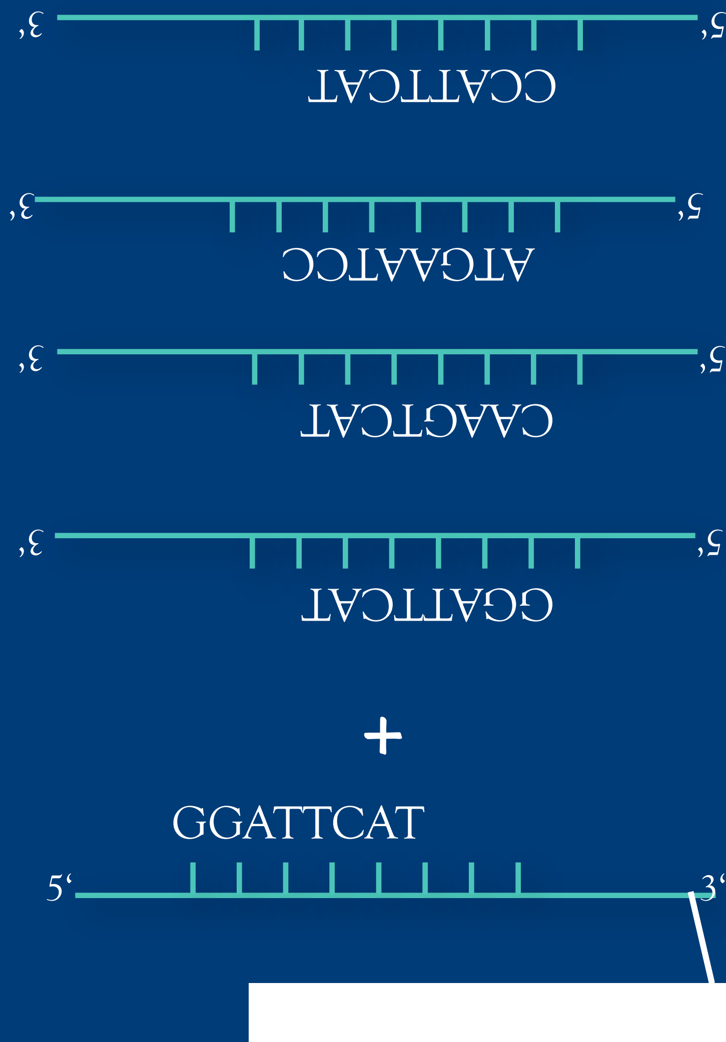
(Teyler TJ et al, Behav. Neurosci, 1986)

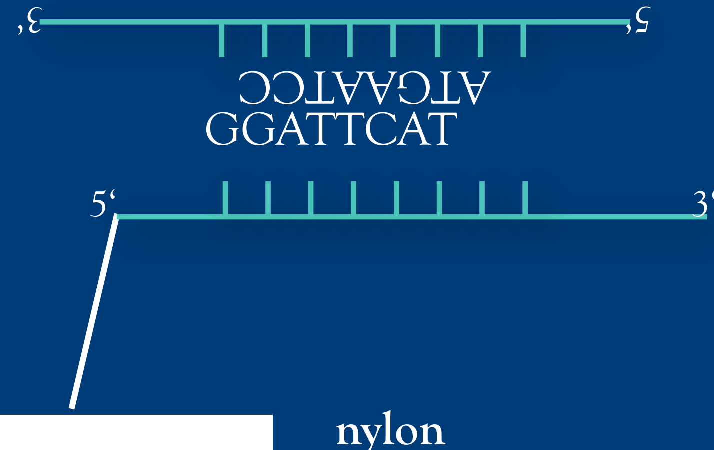
- Hippocampus
  - 記憶鞏固(memory consolidation)
  - 記憶形成(memory formation)

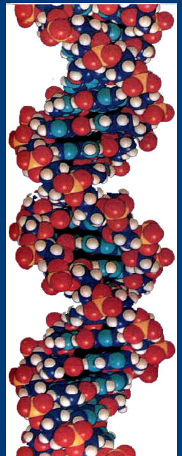
(Morris et al, Neuron, 2006 )

# Hybridization of complementary DNA (cDNA)

雙股DNA序列互補, 經由雜合反應，固定於晶片上的基因可以和它的互補DNA結合，而形成雙股螺旋



nylon

- 阿茲海默症之神經退化與氧化壓力有關
  - 類澱粉樣蛋白沉積→氧化壓力→自由基攻擊→神經病變及神經元喪失。
    - 乙醯膽鹼分泌↓
      - 額葉
      - 巨大基底核 (nucleus basalis of magnocellularis)和內側隔核

(Strada O. *Proc Natl Acad Sci U S A* 1992)

    - 膽鹼乙醯化酶 (choline acetyltransferase)↓
      - 退化程度與痴呆程度成正比

(Pfefferbaum A. Arch Gen Psychiatry 1999 )

# 利用cDNA microarray評估scopolamine對大鼠海馬迴基因表現差異

protocol

- 將cDNA (complementary DNA)放入96孔盤
- 再利用probe (探針)置放於耐龍薄膜的表面
- 取海馬迴mRNA
- 進行雜交(hybridization)反應

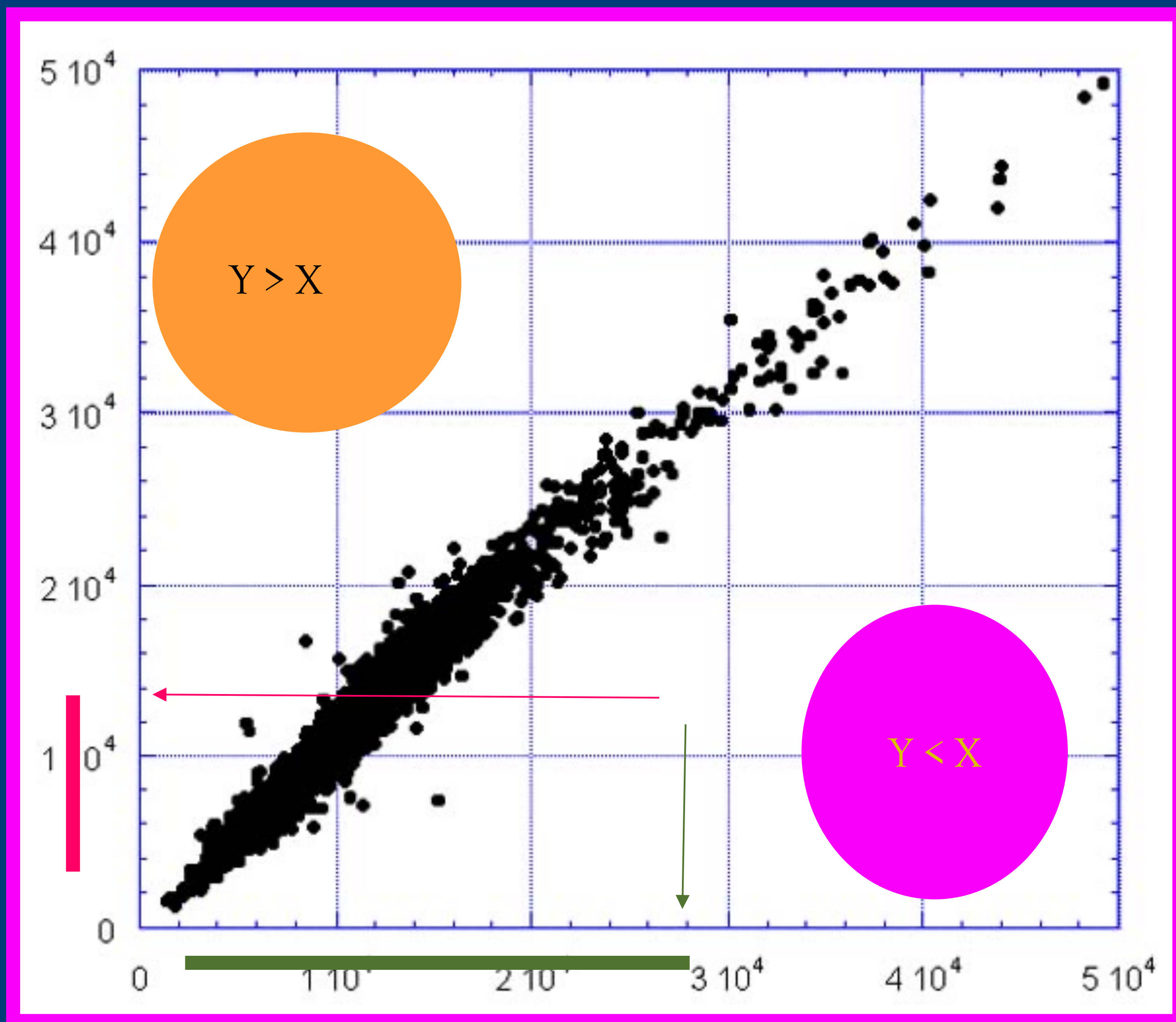



Figure 4. Scatter plot of microarray dataset. Gene expression signal of **SCOP-treated** rat hippocampus (Y-axis) was plotted against counterpart signal of **control** group (X-axis).

|  | *Gene name* |  | *Gene name* |
|---|---|---|---|
| 1 | Presenilin 2 (Alzheimer disease 4) | 25 | Secretory carrier membrane protein 2 |
| 2 | Amyloid precursor-like protein 2 | 26 | Deiodinase, iodothyronine, type II |
| 3 | Amyloid beta (A4) precursor protein-binding | 27 | ATP-binding cassette, sub-family B (MDR/TAP), member 1 |
| 4 | Glutamine synthetase | 28 | ATP-binding cassette, sub-family G, member 1 |
| 5 | nositol 1,4,5-triphosphate receptor | 29 | Protein kinase inhibitor, alpha |
| 6 | Protein tyrosine phosphatase, non-receptor type substrate 1 | 30 | Phosphate cytidylyltransferase 2, ethanolamine |
| 7 | Eukaryotic translation initiation factor 2, subunit 1 | 31 | Calpain large subunit |
| 8 | Epidermal growth factor receptor | 32 | Prostaglandin E synthase |
| 9 | Microtubule-associated protein tau | 33 | Ferredoxin 1 |
| 10 | Protein kinase, interferon-inducible double-stranded RNA | 34 | Cadherin 2, type 1, N-cadherin (neuronal) |
| 11 | Arginase type II | 35 | Aquaporin 5 |
| 12 | cAMP-regulated guanine nucleotide exchange factorI | 36 | Immunoglobulin (CD79A) binding protein 1 |
| 13 | Calcium channel, voltage-dependent, L type, alpha1D subunit | 37 | L1-like cell adhension molecule |
| 14 | Cytochrome c oxidase subunit Vb | 38 | Prolyl endopeptidase |
| 15 | Endothelial differentiation sphingolipid G-protein-coupled receptor | 39 | Asialoglycoprotein receptor 1(hepatic lectin) |
| 16 | Ras-related GTP-binding protein ragA | 40 | Baculoviral IAP repeat-containing 5 |
| 17 | MAP-kinase activating death domain | 41 | Selenoprotein P, plasma, 1 |
| 18 | Programmed cell death 4 | 42 | Phosphoglycerate mutase 1 |
| 19 | Testis-specific heat shock protein-related gene hst70 | 43 | Aquaporin 9 |
| 20 | Coagulation factor C homolog (Limulus polyphemus) | 44 | Neuromedin B receptor |
| 21 | Phosphoglycerate mutase 1 | 45 | Alpha-actinin 1 |
| 22 | Burkitt lymphoma receptor 1 | 46 | Interferon regulatory factor 1 |
| 23 | Glypican 3 | 47 | Epithelial membrane protein 3 |
| 24 | Coronin, actin binding protein 1B | 48 | Phosphate cytidylyltransferase 2, ethanolamin |

# *Validation of microarray datasets by semi-quantitative RT-PCR*

- 為了確認基因晶片所得的結果是否無誤，我們以RT-PCR的技術來加以證明
- 實驗中以ribosomal protein L21 和L18來作標準對照
- 最後得到28個正確無誤的基因

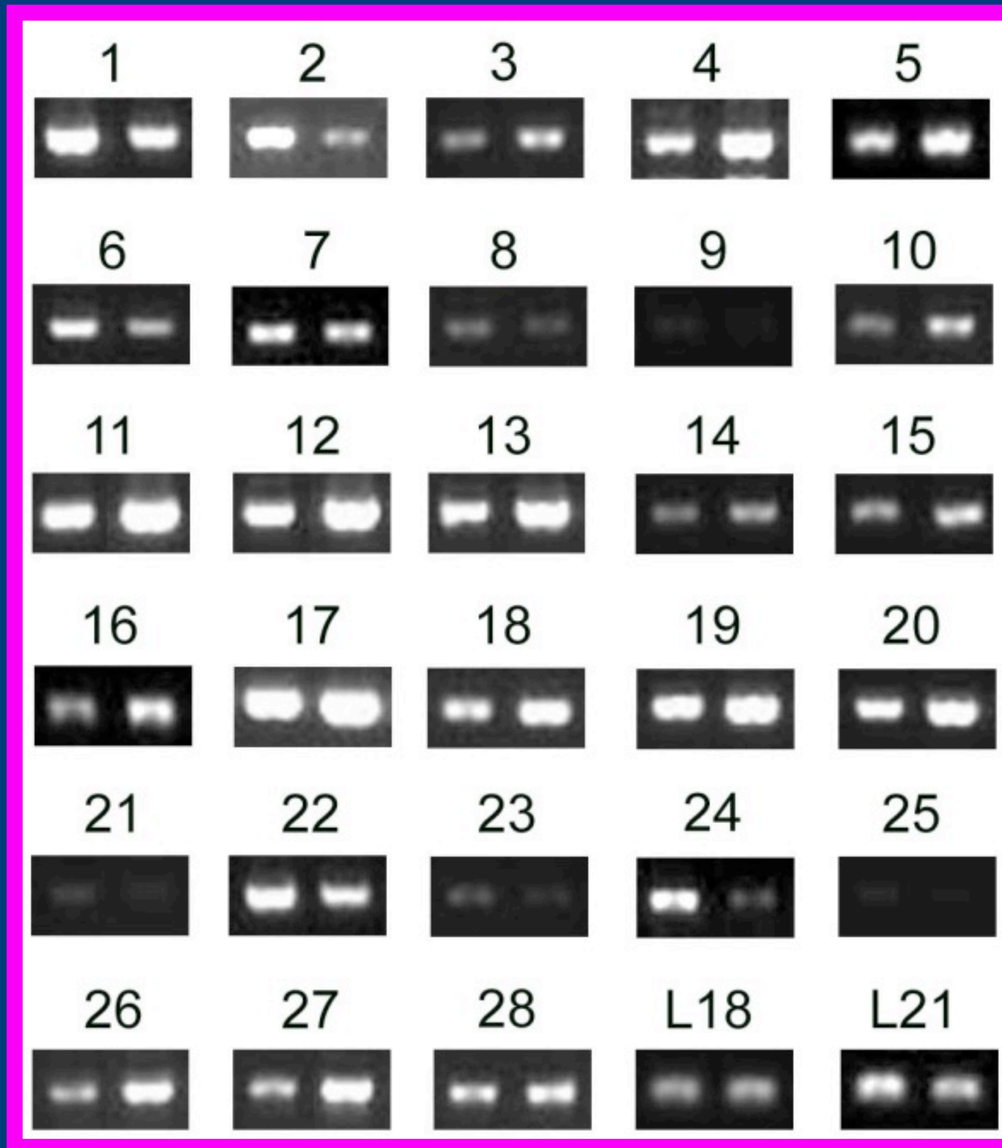



Figure. 6 In each gel slice, left lane is RT-PCR product from scopolamine-treated rat hippocampus, while right lane represents control rat. RT-PCR of ribosomal proteins L21 and L18 were performed as internal controls.

| | Gene Name |
|---|---|
| | ***Alzheimer's disease*** |
| 1 | microtubule-associated protein tau (↓) |
| 2 | presenilin 2 (Alzheimer disease 4) (↓) |
| 3 | ATP-binding cassette, sub-family G, member 1 (Abcg1) ( ↑ ) |
| 4 | amyloid precursor-like protein 2 ( ↑ ) |
| 5 | amyloid beta (A4) precursor protein-binding, family A, member 1 (X11) ( ↑ ) |
| | ***Muscarinic receptor*** |
| 6 | protein kinase, interferon-inducible double-stranded RNA dependent (↓) |
| 7 | arginase type II (↓) |
| 8 | **cAMP-regulated guanine nucleotide exchange factor I (cAMP-GEFI)** (↓) |
| 9 | ATP-binding cassette, sub-family B (MDR/TAP), member 1 (↓) |
| 10 | **calcium channel, voltage-dependent, L type, alpha1D subunit** ( ↑ ) |
| 11 | cytochrome c oxidase subunit Vb ( ↑ ) |
| 12 | endothelial differentiation sphingolipid G-protein-coupled receptor 1 ( ↑ ) |
| 13 | **ras-related GTP-binding protein ragA** ( ↑ ) |
| 14 | **MAP-kinase activating death domain** ( ↑ ) |
| 15 | programmed cell death 4 ( ↑ ) |
| 16 | testis-specific heat shock protein-related gene hst70 ( ↑ ) |
| 17 | glutamine synthetase ( ↑ ) |
| 18 | **inositol 1,4,5-triphosphate receptor** ( ↑ ) |
| 19 | protein tyrosine phosphatase, non-receptor type substrate 1 ( ↑ ) |
| 20 | eukaryotic translation initiation factor 2, subunit 1 ( ↑ ) |
| | ***Other*** |
| 21 | coagulation factor C homolog (Limulus polyphemus) (↓) |
| 22 | phosphoglycerate mutase 1 (↓) |
| 23 | Burkitt lymphoma receptor 1 (↓) |
| 24 | glypican 3 (↓) |
| 25 | epidermal growth factor receptor (↓) |
| 26 | coronin, actin binding protein 1B ( ↑ ) |
| 27 | secretory carrier membrane protein 2 ( ↑ ) |
| 28 | deiodinase, iodothyronine, type II ( ↑ ) |

# 小結 7

## 本研究首次發現經由scopolamine誘發大鼠海馬迴基因表現差異的有

- *Alzheimer's disease*
- *Muscarinic receptor signal transduction*
- *Other*

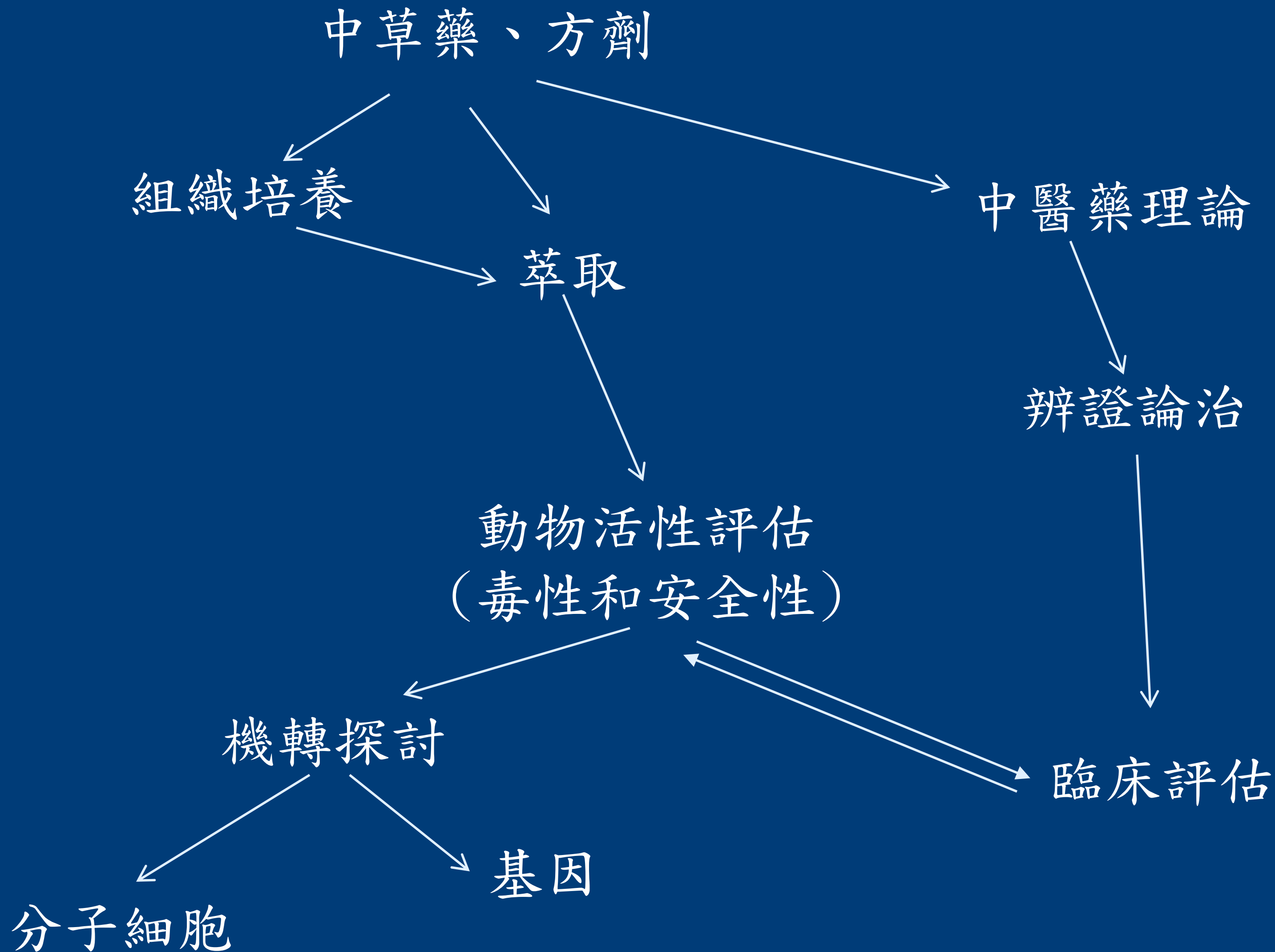
(Hsieh MT, Lin LW et al, *Life Sci*, 2003)

# *Alzheimer's disease*

- Amyloid-beta protein
- Presenilin 2
- Microtubule-associated protein tau
- Amyloid beta precursor protein-binding
- ABC transporter

⇨ 此與*Alzheimer's disease*表現的基因相同

# 中醫藥基礎與臨床研究之趨勢





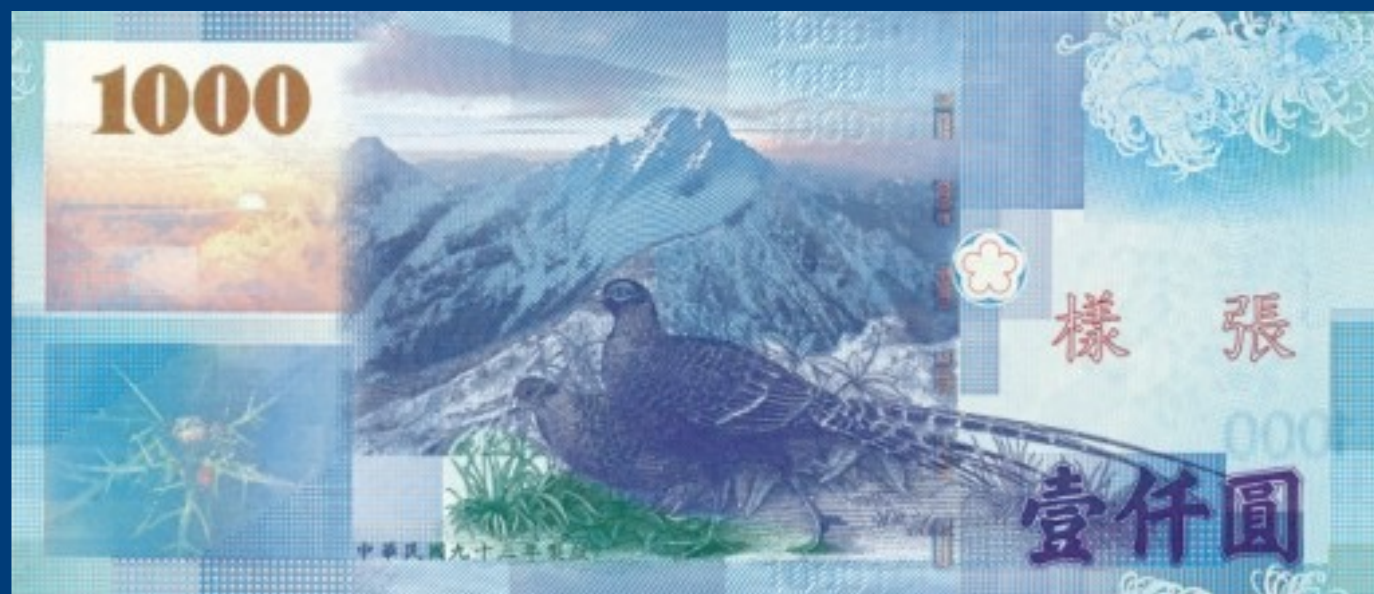



- Review on Experimental Research of Herbal Medicines with Anti-Amnesic Activity. Planta Medica 2010, 76: 203-217.
  - **Ming Tsuen Hsieh**, Wen Huang Peng, Chi Rei Wu, Kit Ying Ng, Chuen Lung Cheng, Hong Xi Xu.

# 結 語

1. 據最近報導：美國國立衛生研究院的輔助與替代醫療研究中心(NCCAM)成立於1992年，經過17年無數的臨床研究後，在中草藥方面沒有發現任何一種有利的結果。
2. 中草藥之臨床療效評估，必須在中醫辨證論治之基礎上，再以現代醫學之臨床評估方法，始能做療效比較研究(Comparative Effectiveness Research) 。


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玉山薊

(川上氏薊)



*Cirsium kawakamii* Hayata

# 大薊





【學名】 *Cirsium japonicum* DC.

【科名】菊科（Asteraceae）

【別名】虎薊、雞腳刺、雞角刺。

【型態】多年生草本。莖直立，有細縱紋，基部具白色絲狀毛。基生葉有柄，葉片倒披針形或倒卵狀橢圓形，羽狀深裂，裂片5～6對，長橢圓狀披針形或卵形，邊緣齒狀，**齒端有尖刺**，上面綠色，疏生絲狀毛，下面灰綠色，脈上有毛；**基部抱莖，羽狀深裂，邊緣有刺。夏季開花，紫紅色。**



【藥用部位】全草或根
【成分】大薊苷(pectolinarin)
【效用】有涼血止血、祛痰、消癰腫、安胎止血、破宿血、生新血之效。為治血要藥，主治吐血、便血、尿血、腸風、癰腫、子宮出血、肝炎、腎炎、跌打損傷、帶狀泡疹、燙風傷、高血壓、骨刺。
【方例】1.治肺癰：大薊4兩，煎湯，早、晚飯後服用。
2.急性肝炎：鮮品4兩，水煎酌加紅糖調服。
3.月經不調、痛經、白帶：本品3～5錢，水酒煎服或月經乾淨前一天，開始連續服用3天。

# 小薊



【學名】 Cirsium setosum(Willd.)MB.

【科名】菊科 (Asteraceae)

【別名】貓薊、青刺薊、千針草、刺薊菜、刺兒菜、青青、小薊姆、刺兒草。

【型態】葉片長橢圓狀披針形，全緣或微齒裂至羽狀深裂，**齒尖具針刺**；上表面綠褐色，下表面灰綠色，兩面均具白色柔毛。頭狀花序單個或數個頂生；**總苞鐘狀，黃綠色；花紫紅色**。



【藥用部位】全草。

【效用】涼血止血，散瘀解毒消癰。用于衄血、吐血、尿血、便血、崩漏下血、外傷出血、癰腫瘡毒。

【方例】1.傳染性肝炎：鮮刺兒菜根狀莖60克.水煎服。

2.功能性子宮出血：鮮刺兒菜60克.水煎分2次服。



昔時賢文

自恨枝無葉

莫怨太陽偏



# 破窯賦・宋　呂蒙正

蜈蚣百足行不及蛇，

家雞翅大飛不及鳥，

馬有千里之程，非人不能自往

